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The farnesyl protein transferase inhibitor SCH66336 synergizes with taxanes in vitro and enhances their antitumor activity in vivo

Abstract Purpose: SCH66336 is an orally active, farnesyl protein transferase inhibitor. SCH66336 inhibits ras farnesylation in tumor cells and suppresses tumor growth in human xenograft and transgenic mouse cancer models in vivo. The taxanes, paclitaxel (Taxol) and docetaxel (Taxotere) block cell mitosis by enhancing polymerization of tubulin monomers into stabilized microtubule bundles, resulting in apoptosis. We hypothesized that anticancer combination therapy with SCH66336 and taxanes would be more efficacious than single drug therapy. Methods: We tested the efficacy of SCH66336 and taxanes when used in combination against tumor cell proliferation in vitro, against NCI-H460 human lung tumor xenografts in nude mice, and against mammary tumors in wap-ras transgenic mice. Results: SCH66336 synergized with paclitaxel in 10 out of 11 tumor cell lines originating from breast, colon, lung, ovary, prostate, and pancreas. SCH66336 also synergized with docetaxel in four out of five cell lines tested. In the NCI-H460 lung cancer xenograft model, oral SCH66336 (20 mg/kg twice daily for 14 days) and intraperitoneal paclitaxel (5 mg/kg once daily for 4 days) caused a tumor growth inhibition of 56% by day 7 and 65% by day 14 compared to paclitaxel alone. Male transgenic mice of the wap-ras/F strain [FVB/N-TgN(WapHRAS)69LInYSIL] spontaneously develop mammary tumors at 6–9 weeks of age which have been previously shown to be resistant to paclitaxel. Paclitaxel resistance was confirmed in the present study, while SCH66336 inhibited growth of these tumors. Most importantly, SCH66336 was able to sensitize wap-ras/F mammary tumors to paclitaxel chemotherapy. Conclusion: Clinical investigation of combination therapy using SCH66336 and taxanes in cancer patients is warranted. Further, SCH66336 may be useful for sensitizing paclitaxel-resistant tumors to taxane treatment.

Key words Farnesyl protein transferase inhibitor · Paclitaxel · Docetaxel · Ras transgenic mice

Introduction

Oncogenic mutations in the ras gene are prevalent in human cancer, including up to 50% of colon cancers and more than 90% of pancreatic carcinomas [1]. In normal cells, RAS switches between an inactive GDP-bound and an active GTP-bound state which can initiate several intracellular signaling pathways [11]. RAS signaling is terminated by hydrolysis of GTP to GDP in a reaction that is stimulated by guanosine triphosphatase-activating proteins. As a consequence of specific mutational events in the ras sequence, oncogenic RAS proteins have a greatly reduced capacity to hydrolyze GTP. This leads to constitutive activation of downstream signaling pathways resulting in unregulated cellular proliferation [1, 19]. Three ras genes encode four ras protein isoforms (H-ras, N-ras, K-ras4A, and K-ras4B) with K-ras4A and K-ras4B being splice variants of the same gene transcript [19]. Although the functional differences between the four isoforms remain unknown, oncogenic mutations of different isoforms predominate in different tumors [2]. H-ras mutations are generally found in carcinomas of the bladder, kidney and thyroid. N-ras mutations are found in myeloid and lymphoid cancers, liver carcinoma and melanoma. K-ras mutations predominate in colon, lung and pancreatic carcinomas.

Many lines of evidence suggest that antitumor activity can be achieved by interfering with the function of
oncogenic RAS proteins [5, 6, 29, 36]. Signal transduction by RAS is dependent on its plasma membrane localization. This localization is supported by a series of post-translational modifications, the first of which is farnesylation of a Cys residue near the C-terminus of RAS proteins. This reaction is catalyzed by farnesyl protein transferase (FPT). RAS prenylation is critical for proper membrane localization and function [9, 12, 34]. Therefore, FPT inhibition is a potential mechanism for interfering with RAS-driven tumor growth.

Prenylation of Ras proteins is complex. In vitro, both K- and N-RAS proteins can serve as substrates for a related protein prenýl transferase, geranylgeranyl protein transferase-I (GGPT-1) [10, 43]. Although this reaction occurs with a lower catalytic efficiency than the farnesylation of these proteins, geranylgeranylation of K- and N-RAS proteins has been observed in cells treated with FPT inhibitors (FTIs) [32, 42]. In contrast, the H-RAS protein is not a substrate for GGPT-1 in vitro or in cells treated with FTIs. Despite this alternative prenylation, FTIs demonstrate in vitro and in vivo antitumor efficacy in a variety of preclinical cancer models [13, 14, 18, 22, 37]. Therefore, the observed activity of FTIs may, in some cases, be due to the inhibition of farnesylation of proteins in addition to or other than RAS.

SCH66336 is an orally active, potent, and selective inhibitor of the FPT enzyme [17, 27]. This novel therapeutic agent has activity against a wide variety of human tumor xenografts and also causes regression of tumors in wap-H-ras transgenic mice. Enhanced antitumor activity has been reported in preclinical cancer models when SCH66336 is combined with cyclophosphamide, 5-fluorouracil, vincristine, and p53 gene therapy [17, 26].

In the studies reported here, we examined the efficacy of SCH66336 in combination with the taxanes, paclitaxel (Taxol) and docetaxel (Taxotere). Taxanes inhibit cell replication by enhancing polymerization of tubulin monomers into stabilized microtubule bundles that are unable to reorganize into the proper structures for mitosis [8, 15, 33]. This results in cell cycle blockage in mitosis and apoptosis, or cell lysis, all of which may be p53-independent [4, 35, 41].

RPMI-1640 (GIBCO) with 10% FBS. AsPC-1 human pancreatic adenocarcinoma cells were cultured in RPMI-1640 (GIBCO) with 20% FBS. MDA-MB-468 human breast adenocarcinoma cells were cultured in Leibovitz’s L-15 medium plus 10% FBS. All the cells were cultured at 37 °C in an atmosphere containing 5% CO₂, except MDA-MB-468 cells which were maintained at 37 °C without CO₂.

In vitro drug interaction studies

SCH66336, (+)-4-[2-[4-(8-chloro-3,10-dibromo-6,11-dihydro-5H-benzo[cyclohepta[1,2-b]pyridin-11-yl]-1-piperidinyl]-2-oxoethyl]-1-piperidinecarboxamide, was synthesized by Schering-Plough and its structure has been published [17, 27]. Paclitaxel (Taxol) was purchased from Calbiochem. Docetaxel (Taxotere, Rhone-Poulenc Rorer) was purchased from Drug Fair (Westfield, N.J.). Paclitaxel and docetaxel were dissolved in absolute ethanol to 10 mg/ml then diluted in culture medium immediately before use. SCH66336, 100 μM in DMSO, was diluted with culture medium for in vitro studies. Tumor cells were seeded into culture wells of 96-well plates and allowed to attach for 3 h. The cells were incubated with paclitaxel or vehicle for 4 h, washed, then SCH66336 or vehicle was added and the incubation continued for 7 days. Multiple dose response assays were generated for each drug alone and in combination, from zero response to maximal response, for each individual cell line. Cell proliferation was measured using the MTT assay [21]. Briefly, 25 μl 5 mg/ml MTT vital dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well and allowed to incubate for 3–4 h at 37 °C in an atmosphere containing 5% CO₂. Then, 100 μl 10% SDS was added to each well and the incubation was continued overnight. Fluorescence in each well was quantitated using a Molecular Devices microtiter plate reader.

Cell proliferation data from drug interaction studies were analyzed using the Thin Plate Spline methodology [28]. Briefly, the response surface (response, dose of A, dose of B) was fitted using a spatial regression model related to thin plate splines. The response is the logit transformation of the percent of cell proliferation [logit = log(percent/100–percent)]. In the model, the dose of A, the dose of B, and the interaction of A and B were the fixed effects. A Gaussian spatial covariance was used for smoothing. The P-value for synergism was given by the P-value for the interaction term in the model.

Computations were performed using the Statistical Analysis System procedure MIXED (SAS/STAT Software, changes and enhancements through release 6.12; SAS Institute, Cary, N.C.). After the spatial regression model fit, the isobole was calculated and graphed using the Statistical Analysis System procedure GRAPH/GRAPHSAS/GRAPH Software, version 6, 1st edn, vol 2. SAS Institute). The smooth response surface was graphed by the procedure G3D.

In vivo efficacy studies

Nude mice (Crl:NU/NU-mBR) were purchased from Charles River Laboratories (Wilmington, Mass.). Line 69-2F wap-ras/F transgenic mice [FvB/N-Tg(WapHRAS;69InYSIL)24, 25] were from the SPRI breeding colony (also available from the Jackson Laboratory Induced Mutant Resource). All mice were maintained in a VAF-barrier facility. Animal procedures were performed in accordance with the Institude for Laboratory Animals and approved by the SPRI Animal Care and Use Committee. SCH66336 was sonicated until dissolved in 20% hydroxypropyl-betacyclodextrin (20% HP/CD). Paclitaxel was dissolved in 100% ethanol, vortexed into Cremophor EL (1/1 v/v; Sigma Chemical Co., St. Louis, Mo.), and diluted into PBS immediately prior to use. Tumor growth was quantitated by measuring tumors in three dimensions. Tumor volumes were calculated as (length × width × height)/2. The statistical significance of tumor growth inhibition in the combination treatment group compared to single-drug treatment on each day was analyzed using Student’s t-test.

Materials and methods

Cell lines

All the human tumor cell lines were purchased from ATCC (Rockville, Md.). MDA-MB-231 human breast adenocarcinoma cells and PANC-1 human pancreatic epitheloid carcinoma cells were cultured in 90% Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, N.Y.) with 10% fetal bovine serum (FBS; Gibco). DU-145 human prostate carcinoma cells and PA-1 human ovarian teratocarcinoma cells were cultured in 90% Eagle’s MEM plus 10% FBS. MIAPaCa2 human pancreatic carcinoma cells were cultured in DMEM with 10% FBS and 2.5% horse serum (HS; Gibco). LNCap human prostate adenocarcinoma cells, DLD-1 human colorectal adenocarcinoma cells, and NCI-H460 human lung large-cell carcinoma cells were cultured in RPMI-1640 (GIBCO) with 10% FBS. AsPC-1 human pancreatic adenocarcinoma cells were cultured in RPMI-1640 (GIBCO) with 20% FBS. MDA-MB-468 human breast adenocarcinoma cells were cultured in Leibovitz’s L-15 medium plus 10% FBS. All the cells were cultured at 37 °C in an atmosphere containing 5% CO₂, except MDA-MB-468 cells which were maintained at 37 °C without CO₂.