European Review for Medical and Pharmacological Sciences 2022; 26: 3726-3734

Molecular insights into the microtubules depolymerizing activity of the IL-8 receptor B antagonist SB225002

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Abstract. – OBJECTIVE: We have previously reported the novel off-target microtubules destabilizing activity of SB225002, a compound that was originally designed as a selective and potent IL-8 receptor B antagonist. In the present study we investigated the reversibility of SB225002 antimitotic effect and provided additional mechanistic insights underlying cell death induction in SW480 human colorectal adenocarcinoma cells.

MATERIALS AND METHODS: Mitotically arrested cells by SB225002 treatment were isolated by shake-off, and their identity was verified by both flow cytometry and immunoblotting. The reversibility of SB225002 antimitotic effects was investigated by flow cytometry and immunoblotting. Prometaphase arrested cells were imaged *via* indirect immunofluorescence and confocal microscopy. Activation of CHK1 in mitotically arrested cells was assessed by immunoblotting, and the relationship between CHK1 and mitotic arrest was examined *via* siRNA-mediated knockdown of CHK1. JNK signaling was evaluated *via* immunoblotting as well as pharmacological inhibition, followed by flow cytometry. The role of reactive oxygen species (ROS) in cytotoxicity was evaluated by ROS scavenging and flow cytometry.

RESULTS: Following SB225002 washout, the mitotic checkpoint was abrogated, and cell cycle perturbations were gradually restored with induction of cell death. Mechanistically, CHK1 checkpoint was activated by SB225002 and occurred downstream of the mitotic checkpoint. In addition, SB225002 activated JNK signaling which contributed to cell death and restrained polyploidy. Furthermore, SB225002 increased intracellular ROS which played a role in mediating SB225002 cytotoxicity.

CONCLUSIONS: Findings of the present study warrants further development of SB225002 as a lead compound that uniquely targets microtubules dynamics and IL-8 signaling.

Key Words:
SB225002, Mitotic arrest, CHK1, JNK, Cell death, Polyploidy, ROS.

Introduction

Targeting the progression of cancer cells during mitosis is an interesting approach for cancer therapy[1,2]. Different classes of compounds have been developed to arrest cancer cells in mitosis, but only tubulin targeting has demonstrated a proven therapeutic feasibility in the clinic[2]. Clinically approved agents, which interfere with microtubules dynamics (either stabilizers or destabilizers), not only directly target cancer cells, but also disrupt their supplying vasculature, which may explain the efficacy of these drugs against a wide range of cancers[3]. Despite that, resistance to antitubulin agents either intrinsic or acquired is a major obstacle that complicates treatment outcomes. Moreover, dose-limiting side effects and/or toxicities may result in poor quality of life or even in treatment discontinuation[2-4]. Therefore, the development of novel antibulin agents is highly valued in the clinic.

We were the first to report the novel and broad-spectrum antitubulin activity of SB225002 against several human cancer cells of different origin, that was mediated *via* interaction with colchicine binding site on the β-tubulin subunit, which ultimately induced cytotoxicity through different mechanisms that are cancer cell type-dependent[5]. Originally, SB225002 was developed as a potent and selective IL8-receptor B antagonist that has the potential to inhibit IL-8-mediated angiogenesis, growth, metastasis and chemoresistance of cancer[6]. Further, we previously showed that the antitumor activity of SB225002 was unaffected by cancer cell overexpression of p-glycoprotein, which is the product of MDR1 gene that frequently imparts drug resistance to the clinically available microtubules inhibitors[3,5]. Moreover, SB225002 was previously reported to exert anti-inflammatory and anti-nociceptive actions *in vivo* through inhibiting the ex-

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pression of iNOS and COX2, along with attenuating pro-inflammatory cytokines, such as IL-1β, TNFα and keratinocyte-derived chemokines[7,8].

Given this promising antitubulin potential of SB225002, the present study was undertaken to shed lights onto the characteristics of SB225002-induced mitotic arrest and to investigate additional mechanisms underlying its cytotoxicity.

Materials and Methods

Chemicals

Both SB225002 and JNK inhibitor SP600125 (Selleckchem, Houston, TX, USA) were dissolved in anhydrous dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA), aliquoted and stored at -80°C. SB225002 aliquots were stored at -80°C for no more than 2 months. Repeated freeze-thaw cycles of aliquots were avoided. N-acetylcysteine was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell Line and Culture Conditions

Human colorectal adenocarcinoma cell line SW480 (ATCC, Mannassas, VA, USA) was maintained in DMEM (Gibco, Grand Island, NY, USA) and were supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), L-glutamine (4 mmol/L for DMEM), 100 units/mL penicillin, and 100 μg/mL streptomycin (Gibco, Grand Island, NY, USA). Cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2.

Immunoblotting

Protein isolation and immunoblotting were carried out as previously described[9]. The primary antibodies used were anti-p-JNK, anti-c-Jun, anti-p-CHK1 ser345, anti-CHK1, anti-Cyclin B1, anti-p-Histone H3, anti-Cdc25c, anti-Securin, anti-β-actin (Cell Signaling Technologies, Beverly, MA, USA); anti-BubR1, (BD Biosciences, San Jose, CA, USA); anti-BcL2 (Dako, Glostrup, Denmark). Secondary antibodies used were HRP-anti-rabbit IgG and HRP-anti-mouse IgG (Cell Signaling Technologies, Beverly, MA, USA).

Analysis of Cell Cycle via Fluorescence-Activated Cell Sorting (FACS)

Cell cycle perturbations were analyzed as previously described[9] using FACSCalibur flow cytometer and CellQuest software package (BD Biosciences, San Jose, CA, USA), with exclusion of cell aggregates *via* FL2W-FL2A gating. A representative data of 3 independent experiments (with triplicate in each) is depicted as the mean±SEM.

Indirect Immunofluorescence and Confocal Microscopy

Immunocytochemistry of α-tubulin was performed as previously described[5], using anti-α-tubulin primary antibody, FITC-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and DNA counterstaining with propidium iodide (Sigma-Aldrich, St. Louis, MO, USA). Olympus FluoView FV1000 confocal microscope (Olympus, Tokyo, Japan) was used for image acquisition and analysis.

Knockdown of Gene Expression via Small Interfering RNA (siRNA)

Knockdown of *CHEK1* gene (encoding CHK1) and scramble siRNA (LacZ) were carried out using previously reported siRNA sequences[10,11] that were synthesized by Qiagen (Hilden, Germany). Cells were transfected using HiPerFect transfection reagent (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Knockdown efficiency was assessed by immunoblotting, and the impact of CHK1 knockdown on cell cycle was assessed by FACS.

Intracellular ROS Assessment

SW480 cells were pretreated with 10 mM N-acetylcysteine for 1 hr, then co-treated with varying concentrations of SB225002 for 12 hours, then cells were processed for loading of CM-H2DCFDA (Invitrogen, Waltham, MA USA) and intracellular ROS were assessed as previously described[9] using FACSCalibur flow cytometer and CellQuest software package (BD Biosciences, San Jose, CA, USA). A representative data of 3 independent experiments (with triplicate in each) is depicted as the mean±SEM.

Statistical Analysis

One-way analysis of variance (ANOVA), followed by Tukey-Kramer post-hoc test, were carried out using SPSS 22.0 Statistics software package (IBM Corp., Armonk, NY, USA). Differences were considered statistically significant at $p < 0.05$.

Results

Mitotic Arrest Induction by SB225002 in SW480 Cells is Reversible and is Associated with Cell Death

To characterize the reversibility of the anti-mitotic activity of SB225002, SW480 cells were

Table I. Flow cytometry-based verification of the identity of SB225002-incued mitotic SW480 cells isolated by shake-off procedures relative to SB225002-treated cells without mitotic shake-off application.

	Cells treated with SB225002 or control vehicle → FACS analysis *WITHOUT* mitotic shake-off		SB225002-treated cells → Mitotic shake-off → FACS analysis of detached and adherent cells	
	DMSO	SB225002	Detached cells	Adherent cells
G1	46.1±4.15	21.2±1.87	2.6±0.23	31.6±2.62
S	34.6±2.77	18.8±1.67	3.6±0.3	28.9±2.25
G2M	19.42±1.84	60.1±5.89	93.4±7.38	39.3±3.03

Results are presented as the mean±SEM. DMSO: Dimethylsulfoxide control vehicle.

treated with SB225002 (650 nM) for 12 hours. The reason why this concentration of SB225002 and the exposure time were adopted is that in our previous work the IC_{50} of SB225002 was approximately 650 nM and mitotic arrest peaked after 12 hours using the same cell line[5]. Then, mitotic shake-off was carried out to separate mitotic cells from interphase cells, and the identity of the isolated cells *vs.* adherent cells was verified by both flow cytometry and immunoblotting. Cell cycle analysis by fluorescence-activated cell sorting (FACS) showed that, following SB225002 treatment, approximately 93% of the separated cells were G2/M arrested (Table I), while the remaining adherent cells were distributed in G1, S and G2/M phases (Table I). These results when compared to those obtained with FACS analysis of SB225002-treated cells without shake-off, clearly revealed the enrichment of G2/M population of the isolated cells (Table I). Moreover, immunoblotting of different mitotic markers showed that the substrates of the anaphase promoting complex cyclin B1 and hyperphosphorylated securin were evidently accumulated in the isolated cells relative to the adherent cells (Figure 1A). Similarly, BubR1 (the mitotic checkpoint kinase), Cdc25c (the mitotic trigger), histone H3 and BcL2 were all strongly hyperphosphorylated in the isolated cells in contrast to adherent cells (Figure 1A), indicating that the isolated cells were mitotically arrested. Collectively, these results confirmed the efficiency of the applied shake-off procedures in isolating mitotic cells following SB225002 treatment.

Next, we assessed the reversibility of SB225002-induced mitotic arrest using the mitotic cells isolated by shake-off procedures. To this end, SB225002 treatment was performed for 12 hours, followed by mitotic cells separation and re-plating into a fresh culture medium (without SB225002), at various time points (2, 4, 8, 16, 24 hours), then cells were harvested for cell cycle analysis by flow cytometry, as well as for the assessment of mitotic markers expression by immunoblotting. Results showed that 2 hours after release, about 80% of the cells were in the G1 phase, 4% appeared as SubG1 population, and the remaining were in S and G2/M phases (Figure 1B). At the subsequent time points, there were time-dependent increments in SubG1 population along with a gradual redistribution of cells among different phases of the cell cycle (Figure 1B). Immunoblotting further supported flow cytometry results where all the examined mitotic markers were normalized 2 hours after release suggestive of a mitotic exit (Figure 1C), and the expression pattern of the assessed mitotic markers at subsequent time points coincided with the corresponding changes in cell cycle distribution in a timely manner (Figure 1B, 1C). Taken together, these findings suggested that after a brief (12 hours) exposure to SB225002 followed by a washout, the mitotically arrested cells can re-enter the cell cycle and undergo cell death, which indicated the reversibility of SB225002-induced mitotic arrest in SW480 cells.

SB225002 Activated CHK1 DNA Damage Checkpoint which Occurred Downstream of the Mitotic Checkpoint in SW480 Cells

We have previously shown that SB225002 triggered a prometaphase arrest and activated the spindle assembly checkpoint (SAC), as a result of microtubules depolymerization and the resultant chromosomal misalignment[5]. Accordingly, we investigated whether SB225002 may activate DNA damage checkpoint in mitotically arrested cells. Confocal microscopy showed the existence of a fragmented chromosomal DNA in prometaphase arrested SW480 cells, following 12 hours of SB225002 treatment (Figure 2A). Time-course immunoblot analysis of DNA damage checkpoints revealed that CHK1 was phosphorylated at serine 345 residue by SB225002 (650 nM) as

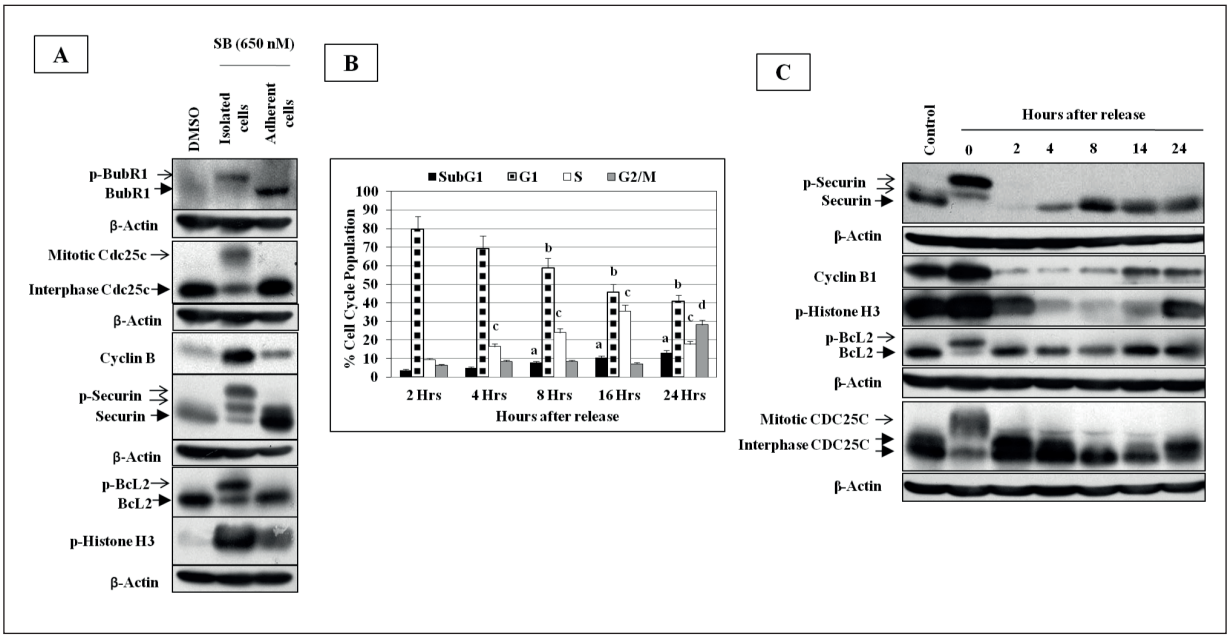

Figure 1. SB225002-triggered mitotic arrest is reversible with induction of cell death in SW480 cells. **A**, Immunoblotting of mitotic markers in SW480 cells treated with SB225002 (650 nM) for 12 hrs, then subjected to mitotic shake-off. Loading control: β-Actin. **B**, Time-course FACS analysis, and **C**, Time-course immunoblotting of mitotic markers. Both **B** and **C** were carried out on the isolated mitotic SW480 cells after being treated with SB225002 (650 nM) for 12 hrs, then released into fresh culture medium for 2, 4, 8, 16, 24 hrs. The small letters a, b, c, d in panel **B** indicate statistically significant differences from the corresponding cell cycle population of the 2 hrs time point at $p < 0.05$.

early as 1.5 hour, and this phosphorylation peaked after 12 hours, but declined thereafter (Figure 2B) coinciding with the kinetics of hyperphosphorylation of a prominent mitotic marker securin (Figure 2B). On the other hand, CHK2 phosphorylation could not be detected (data not shown). Further, CHK1 serine 345 phosphorylation occurred in a dose-dependent manner following SB225002 treatment for 12 hours (Figure 2C). Importantly, mitotic shake-off that was carried out on SW480 cells – treated with SB225002 for 12 hours – and was followed by immunoblotting, showed that CHK1 serine 345 phosphorylation was confined to the isolated mitotic cells, but was undetectable in the adherent interphase cells (Figure 2D). These findings suggested that the activation of CHK1 DNA damage checkpoint exclusively occurred in the mitotically arrested SW480 cells, following SB225002 treatment.

Furthermore, we investigated whether CHK1 knockdown may influence mitotic markers expression by immunoblotting. Results showed that an efficient CHK1 depletion was unable to abrogate SB225002-induced accumulation of cyclin B1 and hyperphosphorylated securin (Figure 3A). Similarly, Cdc25c, histone H3 and BcL2 retained their mitotic hyperphosphorylation upon SB225002 treatment and CHK1 knockdown (Figure 3A). Moreover, flow cytometry revealed that CHK1 knockdown failed to abrogate mitotic arrest, triggered by SB225002 (Figure 3B). Collectively, these findings suggested that the activation of CHK1 DNA damage checkpoint occurred downstream of the mitotic arrest induced by SB225002.

SB225002 Activated JNK Signaling in SW480 Cells

Given that microtubules inhibitors can activate p38MAPK and/or JNK[12], and that our previous findings showed the activation of p38MAPK signaling by SB225002[5], we investigated whether SB225002 may increase the phosphorylation of JNK in SW480 cells. Immunoblotting revealed that SB225002 dose-dependently upregulated JNK phosphorylation, following 12 hours of treatment (Figure 4A). Further, treatment with SB225002 brought about a dose-dependent upregulation of JNK downstream target c-Jun (Figure 4A). These findings indicated that JNK signaling was activated by SB225002 in SW480 cells.

To gain insights into the functional consequences of JNK activation by SB225002, we

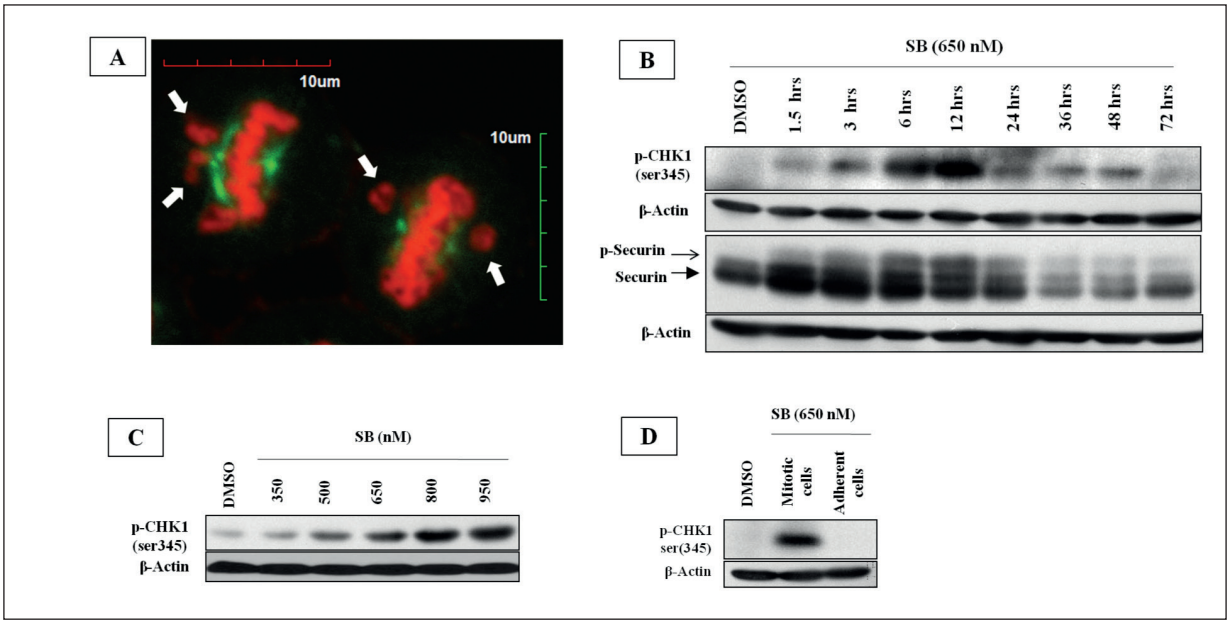

Figure 2. Activation of CHK1 DNA damage checkpoint by SB225002 in SW480 cells. **A**, Confocal microscopy of tubulin immunocytochemistry in SW480 cells treated with SB225002 (650 nM for 12 hrs). Green: tubulin, Red: DNA. Arrows indicate fragmented misaligned DNA. Scale bar=10 μm. **B**, Time-course immunoblotting of phosphorylated CHK1 and securin in SW480 cells treated with SB225002 (650 nM). **C**, Immunoblotting of phosphorylated CHK1 in SW480 cells treated with different concentrations of SB225002 for 12 hrs. Loading control: β-Actin. **D**, Immunoblotting of phosphorylated CHK1 in isolated mitotic *vs.* adherent SW480 cells treated with SB225002 (650 nM) for 12 hrs. Loading control: β-Actin.

challenged SW480 cells with a well-known JNK inhibitor, prior to SB225002 treatment and immunoblotting was used to validate the efficiency of JNK signaling inhibition. Results revealed a marked abrogation of c-Jun upregulation by SB225002 (Figure 4B). Next, FACS analysis of

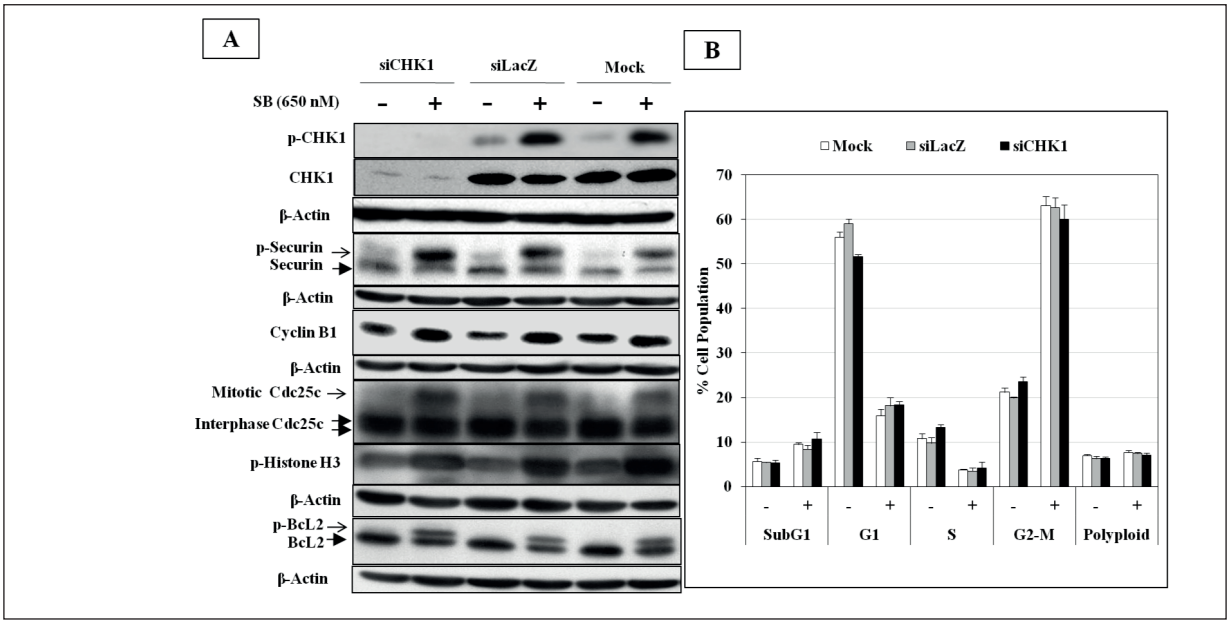

Figure 3. SB225002-induced CHK1 DNA damage checkpoint occurred downstream of the mitotic checkpoint in SW480 cells. **A,** Immunoblotting of the expression of mitotic markers in SW480 cells upon knockdown of CHK1 followed by treatment with SB225002 (650 nM) for 12 hrs. Loading control: β-Actin. **B**, FACS analysis of SW480 cells following CHK1 knockdown and SB225002 treatment (650 nM, 12 hrs).

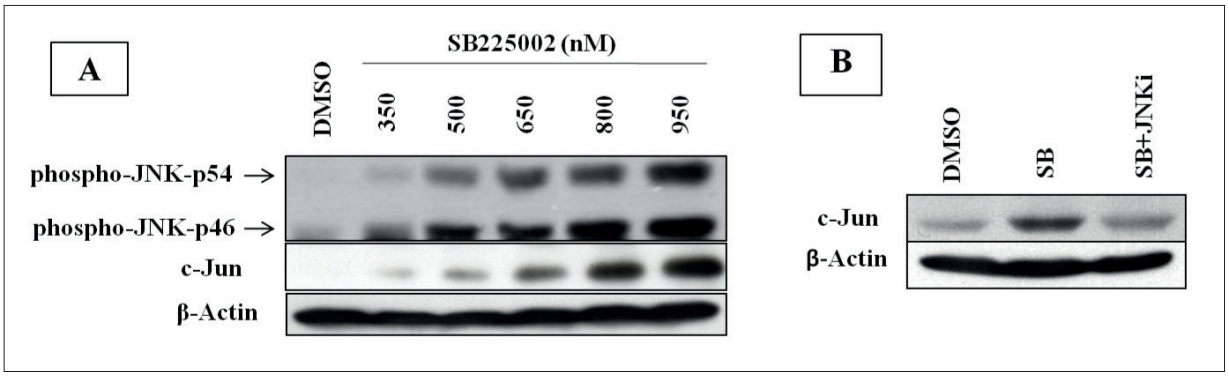

Figure 4. SB225002 activated JNK signaling in SW480 cells. ***A***, Immunoblotting of phosphorylated JNK and its downstream target c-Jun in SW480 cells treated with different concentrations of SB225002 for 12 hrs. Loading control: β-Actin. **B**, Immunoblotting of c-Jun in SW480 cells pre-treated with JNK inhibitor SP600125 (10 μM) for 1 hr then co-treated with SB225002 (650 nM) for 12 hrs. Loading control: β-Actin.

SW480 cells treated with SB225002 in the presence or absence of the JNK inhibitor showed that SB225002-induced SubG1 population was significantly inhibited by JNK inhibitor pretreatment, while polyploid cells were remarkably and dose-dependently increased by SB225002/JNK inhibitor co-treatment (Table II). These findings suggested that following SB225002 treatment, JNK activation contributed to cell death and restrained polyploid cell formation.

Intracellular ROS Contributed to SB225002-Induced Cell Death

Given that mitotically arrested cells were shown to generate excessive intracellular ROS which could mediate cell death[13], we investigated their potential role in SB225002 cytotoxicity. Flow cytometry analysis of intracellular ROS revealed that their levels were dose-dependently elevated by SB225002 treatment (Table III). Moreover, pretreatment with N-acetylcysteine efficiently scavenged SB225002-induced intracellular ROS accumulation (Table III). Next, we investigated the effect of N-acetylcysteine pretreatment on SB225002-induced cell death by FACS analysis. Results showed that ROS scavenging significantly abrogated SB225002-induced SubG1 population and largely normalized different phases of the cell cycle (Table IV). These findings suggested that ROS contributed, at least in part, to the cytotoxicity induction by SB225002.

Discussion

Microtubules have been established as a valid target in cancer treatment, driving the continual interest in the introduction of novel antitubulin agents with enhanced efficacies and/or reduced side effects[3,4].

The reversibility of tubulin targeting is usually associated with a better tolerability and lower

Table II. Flow cytometry analysis of the influence of JNK inhibitor pre-treatment on SB225002-induced cell cycle perturbations and cell death of SW480 cells.

	SubG1		G1		S		G2/M		Polyploid	
		+JNKi		+JNKi		+JNKi		+JNKi		+JNKi
DMSO	1.5±0.14	1.8±0.16	57.2±5.15	54.2±4.72	19.7±1.77	20.9±1.88	18.2±1.44	19.8±1.8	2.7±0.24	3.2±0.27
SB350	5.1±0.18	1.7±0.35*	53.1±4.78	45.6±3.71*	16.5±1.66	14.8±1.2	21.1±1.79	24.8±2.1*	3.8±0.33	11.9±1.01*
SB500	14.9±0.63	5.8±0.46*	39.8±3.54	19.1±1.53*	12.1±1.29	10.6±1.1	24.6±2.21	30.5±2.4*	7.8±0.68	33.1±2.65*
SB650	19.3±1.01	8.1±0.62*	25.3±2.13	15.2±1.16*	13.1±1.3	8.8±0.67*	26.5±2.74	25.2±1.92	15.4±1.17	42.2±3.63*
SB800	21.4±1.52	11.1±0.79*	15.7±1.22	11.1±0.79*	11.1±0.87	8.2±0.58*	23.7±2.32	20.9±1.48	27.4±1.95	47.9±4.55*
SB950	24.5±2.33	16.4±1.45*	11.1±0.96	8.2±0.7*	8.5±0.81	5.8±0.65	20.1±1.71	18.8±1.79	34.8±2.69	49.9±3.24*

Results are presented as the mean±SEM. Asterisk indicates a statistically significant difference at $p < 0.5$ of JNK inhibitor/SB225002 co-treated cells versus SB225002-only treatment. SB: SB225002; JNKi: JNK inhibitor; DMSO: Dimethylsulfoxide control vehicle.

Table III. Investigating the effect of SB225002 on intracellular ROS generation by flow cytometry in SW480 cells (in the presence or absence of N-acetylcysteine pre-treatment).

	Intracellular ROS *[Median Fluorescence Intensity]*	
		+ NAC
DMSO	7.5±0.75	7.2±0.71
SB350	9.5±0.69	7.5±0.62
SB500	14.2±1.4	8.4±0.81*
SB650	16.3±1.2	9.8±0.79*
SB800	18.4±1.3	10.6±0.92*
SB950	19.9±1.4	11.4±1.1*

Results are presented as the mean±SEM. Asterisk indicates a statistically significant difference at $p<0.5$ of N-acetylcysteine/SB225002 co-treated cells versus SB225002-only treatment. SB: SB225002; NAC: N-acetylcysteine; DMSO: Dimethylsulfoxide control vehicle

grade dose-limiting side effects such as myelosuppression or peripheral neuropathy which are commonly observed with the clinically available microtubules inhibitors[4,14]. In the present study, we demonstrated that the antimitotic activity of SB225002 is reversible, which may be seen as a potential therapeutic merit for this compound.

Pharmacologic induction of a prolonged mitotic arrest has been shown to result in DNA breaks in mitotic chromosomes and activation of DNA damage checkpoints which preceded cells death[15]. Our results demonstrated the existence of chromosomal DNA breaks in prometaphase arrested SW480 cells, and the increased serine 345 phosphorylation of CHK1 DNA damage checkpoint specifically in mitotic cells following SB225002 treatment. Similarly, phosphorylation of CHK1 at serine 345 was previously shown to occur at DNA damage foci in stressed cells undergoing mitosis, and phosphorylation at this residue is a critical regulator of CHK1 kinase activity[16-18].

Previous reports have suggested that modulation of CHK1 expression and/or phosphorylation can either trigger the spindle assembly checkpoint (SAC) or may occur as a downstream event of SAC activation[19,20]. Further, the role of CHK1 during mitotic arrest varies depending on the type of the antitubulin agent where CHK1 activity was required for SAC activation and mitotic arrest induction by microtubules stabilizers[20]. Conversely CHK1 was dispensable for the antimitotic activity of microtubules depolymerizes[20]. Our findings have shown that CHK1 activation by SB225002 is a downstream event of mitotic arrest induction. Importantly, the knockdown of CHK1 did not affect the accumulation of mitotic markers or cell cycle arrest at the M-phase, which further supported our previously reported mechanism of SB225002 as a microtubule depolymerizer[5].

Microtubules inhibiting agents have been shown to robustly induce JNK phosphorylation in human cancer cells[21,22], and JNK activation has been shown to link prolonged mitotic arrest to cell death[23]. Furthermore, the contributions of JNK toward mitotic progression through anaphase and protection against aneuploidy have previously been suggested where the inhibition of JNK activity could inhibit chromosome segregation during anaphase and induced polyploidy[24,25]. Polyploid cancer cells, which are commonly formed due to the prolonged activation of mitotic checkpoint, have a number of criteria such as: expression of cancer stem-like cell markers, resistance to treatment, protection against host immune system, and the capacity to undergo de-polyploidization to re-initiate tumor growth[1,26,27]. Therefore, it may be concluded that the activation

Table IV. Flow cytometry analysis of the impact of SB225002-induced ROS generation on cell cycle perturbations and cell death of SW480 cells.

	SubG1		G1		S		G2/M		Polyploid	
		+NAC		+NAC		+NAC		+NAC		+NAC
DMSO	1.8±0.15	1.9±0.11	58.8±5.77	58.9±5.3	17.9±1.8	18.2±1.5	17.7±1.9	18.3±1.6	2.9±0.25	2.3±0.21
SB350	6.7±0.6	4.4±0.33	54.2±4.88	56.9±5.54	14.1±1.3	15.9±1.6	20.2±1.8	18.6±1.7	4.2±0.38	3.2±0.26
SB500	16.3±1.74	8.2±0.66*	40.6±3.95	53.2±4.3*	11.2±1.1	14.2±1.2*	25.1±1.9	20.3±1.5*	6.4±0.58	3.6±.032*
SB650	21.8±2.14	11.8±1.06*	24.1±2.1	46.1±4.2*	12.3±0.93	13.9±1.3	27.8±1.7	23.1±1.6*	13.5±0.94	4.2±0.42*
SB800	23.4±2.28	12.8±1.11*	17.5±1.85	40.9±3.6*	9.1±0.82	11.8±1.1*	26.1±1.8	22.5±1.7*	23.4±1.47	8.2±0.71*
SB950	25.7±1.52	12.6±1.31*	15.1±1.1	38.3±2.9*	6.7±0.26	9.2±0.65*	21.8±1.5	22.9±1.5	30.9±1.88	12.3±0.93*

Results are presented as the mean±SEM. Asterisk indicates a statistically significant difference at $p < 0.5$ of N-acetylcysteine/SB225002 co-treated cells versus SB225002-only treatment. SB: SB225002; NAC: N-acetylcysteine; DMSO: Dimethylsulfoxide control vehicle.

of JNK signaling by SB225002 in the current study may have two important consequences: contributing to cell death induction and curbing polyploidy, since co-treatment of SW480 with a selective JNK inhibitor, followed by SB225002, significantly promoted polyploidy and abrogated cell death.

The activation of both JNK and p38MAPK by SB225002 treatment in the current study and in our previous work[5] suggested the stressful nature of the mitotic arrest triggered by SB225002, which was further supported by its potential to induce ROS generation demonstrated in the present study. Likewise, prolonged mitotic arrest induction has previously been shown to exacerbate ROS generation[13]. Recently, mitochondrial dysfunction was shown to occur during mitotic arrest caused by microtubules inhibiting agents, resulting in oxidative stress that contributed to the antitumor activity of chemotherapeutics that target the mitotic phase[28].

Conclusions

In summary, the molecular mechanisms, and the distinctive properties of SB225002 described thus far provided a strong rationale for further development of SB225002 as a lead antitumor agent that uniquely targets microtubules dynamics and IL-8 signaling.

Authors' Contribution

Goda AE: Project conceptualization. Methodology, data curation, and analysis. Writing- draft and editing. Writing- final review and approval. Sakai T: Methodology, data curation, and analysis. Writing- final review and approval.

Conflict of Interest

The authors declare no known competing financial interests or personal relationships that may have influenced this paper in whole or in part.

Funding received

None.

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