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Differential Sensitivities of Human Multidrug-resistant Cancer Cells to BIIB021 and Modulation of Hsp90 Inhibitors by NSAIDs and Niclosamide

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The critical role of heat shock protein 90 (Hsp90) in tumorigenesis led to the development of several first- and second-generation Hsp90 inhibitors, which have demonstrated promising responses in cancers. In this study, we found second-generation Hsp90 inhibitor BIIB021-resistant multidrug-resistant (MDR) human cancer cells, although BIIB021 was shown to be active in first-generation Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG)-resistant MDR cells. MCF7-MDR and HeyA8- MDR cells were more resistant to BIIB021 than their parental counterparts, indicating that BIIB021 cannot be applicable to all cancer cells expressing MDR proteins. We revealed that dimethyl-celecoxib (DMC), one of the non-steroidal anti-inflammatory drugs (NSAIDs), potentiated cytotoxicity of BIIB021 against both BIIB021-resistant and BIIB021-sensitive MDR cells. The effectiveness of NSAIDs involving celecoxib and DMC in combination with BIIB021 led to the autophagic degradation/down-regulation of mutant p53 (mutp53) that overexpressed MDR cells and the suppression of Hsp70 induction. This resulted in sensitization of MDR cells to BIIB021. Moreover, autophagy induction by sulindac sulfide, another type of NSAID, and niclosamide, an FDA-approved anthelmintic drug, potentiated 17-AAG-mediated autophagic degradation/down-regulation of mutp53 and c-Myc, client proteins of Hsp90. Therefore, our results suggest that NSAIDs and niclosamide positively enhance the anticancer activity of Hsp90 inhibitors through an autophagic pathway. They may also be new candidates for sensitizing MDR cells to Hsp90 inhibitors.

Key words : Autophagy, BIIB021, Hsp90 inhibitor, NSAIDs, Niclosamide

Introduction

Heat shock proteins (Hsps) are molecular chaperones that are consistently increased to help cells survive under conditions of stress. As a member of the Hsps, Hsp90 is a molecular chaperone that plays an important role in the modification and stabilization of a variety of proteins implicated in tumor cell proliferation and survival. Particularly, Hsp90 is unexpectedly abundant to maintain levels of proteins in cancer cells. Hsp90 inhibitors can target the ATP domain of Hsp90 and prohibit its exchange of ADP for ATP, leading to the degradation of client proteins and disruption of signaling cascades. Many of the known clients are protein kinases or transcription factors involved in multiple signal transduction pathways including tyrosine kinases; Bcr-Abl, epidermal growth factor receptor family members, c-Met, insulin-like growth factor-1 receptor and pp60c-src, serine/threonine kinases; Akt, Cdk4, Raf-1 steroid hormone receptors, p53, Stat3, Mdm2 and telomerase. Concomitantly, Hsp90 inhibitors induce tumor cell apoptosis, promote cell cycle arrest and abrogate microenvironmentderived cytoprotection [18].

Geldanamycin and its derivative, 17-allylamino-17-demethoxy-geldanamycin (17AAG), were first developed as Hsp 90 inhibitors (first-generation Hsp90 inhibitors), and they exhibited effective anticancer potency but severe side effects and low solubility restricted their application at the clinical level. Moreover, the antitumor activity of 17-AAG and other ansamycins are significantly curtailed by the expression of multidrug resistance (MDR) proteins, a major contributor to drug resistance commonly observed in heavily pretreated cancer patients. Many types of cancer express relatively high levels of P-glycoprotein (P-gp), a major type of MDR protein, which include colon, kidney, adrenocortical and hepatocellular cancers whereas breast, lung, neuroblastoma and other

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tumors express P-gp at intermediate levels [1, 5]. In many cases, untreated tumors, which do not express MDR proteins, could be induced to express P-gp and other MDR proteins to a high level upon treatment with drugs that are P-gp substrates, including chemotherapeutic drugs and molecularly targeted compounds [4, 15]. Previous studies have shown that the 17-AAG and ansamycin derivatives of Hsp90 inhibitors are inactive in P-gp/MDR1- and/or MRP1-expressing cell lines [16, 19]. 17-AAG might be transported by P-gp in a distinct manner or that the magnitude of the influence of P-gp on ansamycin activity may be related to the slow kinetics of action of these Hsp90 inhibitors in cells.

Second-generation Hsp90 inhibitor BIIB021 is an orally available, fully synthetic novel small-molecule Hsp90 inhibitor that has shown strong antitumor activities in a large number of preclinical models and is now under clinical investigation [3, 14]. It has been reported that over-expression of P-gp is associated with resistance to 17-AAG but not to BIIB021, and BIIB021 is active in P-gp and/or MRP-1 expressing drug-resistant cancer cells [19]. Moreover, BIIB021 potentiate the effects of other therapeutics beyond degrading oncogenic protein [7]. Since the cytotoxicity of BIIB021 in MDR cells showed no correlation with P-gp level in only two tested MDR variants [19], it is necessary to test various MDR cells derived from different types of tumors. In this study, we determined whether BIIB021 could be active against various MDR cells derived from different types of tumors, and also developed new sensitizers that enhance cytotoxicity of Hsp90 inhibitors and thus reverse Hsp90 inhibitor resistance of MDR cells.

Materials and Methods

Cell culture and reagents

Human MDR variants such as CEM/VLB_{100} cells isolated from CEM human lymphoblastic leukemia cells, MCF7-MDR cells isolated from human breast cancer MCF-7 cells and HeyA8-MDR cells isolated from HeyA8 human ovarian cancer (moderately differentiated papillary cystadenocarcinoma of the ovary) cells were used, which were kindly provided by Dr. Fiedler (MD Anderson, TX, USA). These cells were maintained in DMEM supplemented with 10% fetal bovine serum and were incubated at 37℃, 5% CO_2 and 95% humidity. 17-allylamino-17-demethoxy-geldanamycin (17-AAG) (Enzo Life Sciences Inc., Farmingdale, New York, USA), celecoxib (CCB), 2,5-dimethyl-celecoxib (DMC) (Sigma-Aldrich, St. Louis, MO, USA). BIIB021 (Selleckchem, Houston, TX, USA), niclosamide and sulindac sulfide (Sigma-Aldrich, St. Louis, MO, USA) were used in this study.

Cell proliferation assay

Cell proliferation was measured by counting viable cells by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric dye-reduction method. Exponentially growing cells (1- or 2×10^4 cells/well) were plated in a 96-well plate and incubated in growth medium treated with the indicated concentration of BIIB021 and DMC (or CCB) at 37℃. After 96 hr, the medium was removed using centrifugation, and MTT-formazan crystals solubilized in 100 μl DMSO. The optical density of each sample at 570 nm was measured using ELISA reader. The optical density of the medium was proportional to the number of viable cells. Inhibition of proliferation was evaluated as a percentage of control growth (no drug in the medium). All experiments were repeated in at least two experiments in triplicate.

Western blot analysis

Cell lysates from control and indicated drug-treated cells (1×10^6 cells) were prepared using M-PER Reagent (Thermo Scientific Inc., USA). The lysates were clarified by centrifugation. Equal amounts of protein were resolved by SDS-PAGE and immunoblotted with indicated antibodies. Western blot analysis was performed with specific primary antibodies against LC3 and p62 (Novus Biologicals, Littleton, CO, USA), b-actin (Sigma-Aldrich, St. Louis, MO, USA), Hsp70 (Enzo Life Sciences, Inc., NY, USA), c-Myc (Cell Signaling Technology Inc., Beverly, Massachusetts, USA), p53 (Santa Cruz Biotechnology, CA, USA), The p53 antibody (DO-1) is a mouse monoclonal antibody raised against amino acids 11-25 of p53 of human origin (Santa Cruz Biotechnology), which was recommended for detection of wild and mutant p53 of human origin.

Apoptosis assessment by Annexin V staining

The effects of BIIB021 and/or CCB (or DMC) on apoptosis were measured by Annexin V staining. MCF7-MDR cells (2×10^5 cells/ml) were treated with BIIB021 in the presence or absence of CCB and/or DMC for 24 hr. Then cells were centrifuged and resuspended in 100 μl of the staining solution containing Annexin V-fluorescein (FITC Apoptosis detection kit; BD ParMingen San Diego, CA, USA) and propi-

dium iodide (Sigma-Aldrich, St. Louis, MO, USA) in a Hepes buffer. After incubation at room temperature for 20 min, the percentage of early (annexin V positive/PI negative) and late apoptotic cells (annexin V positive/PI positive) was quantified by FACS for Annexin-V and PI staining.

Statistical analysis

A Student's *t*-test was used to calculate the statistical significance of the experimental data and the level of significance was set as $*p<0.05$, $**p<0.01$ and $***p<0.001$.

Results

Differential responses of three MDR cells to Hsp90 inhibitor BIIB021 and enhancement of BIIB021 sensitivity by NSAIDs

Since previous study showed that BIIB021 was active in two MDR variants such as NCI/ADR-RES and MES-SA DX5 cells [19], we therefore examined the changed cytotoxicity of BIIB021 in three MDR variants isolated from tumors of various types such as MCF7-MDR cells, HeyA8-MDR cells and CEM/VLB_{100} cells when compared to BIIB021 cytotoxicy of their parental counterparts. Our data showed that two variants of MDR such as MCF7-MDR and HeyA8-MDR cells were more resistant to BIIB021 than their parental counterparts (Fig. 1A, Fig. 1B), whereas BIIB021 sensitivity of both CEM/VLB_{100} and its parental counterpart was not different, and BIIB021 was active against both cells (Fig. 1C), indicating it is necessary to establish methods for sensitization of BIIB021-resistant MDR cells.

Previous we reported that sensitivity of Hsp90 inhibitor 17-AAG was enhanced by celecoxib (CCB) and its analog 2,5-dimethyl-celecoxib (DMC), non-steroidal anti-inflammatory drugs (NSAIDs) class, via autophagic inducing ability, which resulted in autophagic degradation of mutant p53 (mutp53) that over-expressed MDR cells. [12]. Therefore, we examined whether BIIB021 cytotoxicity in MDR cells could be modulated by DMC. Our data showed that DMC potentiated the cytotoxicity of BIIB021 in BIIB021-sensitive CEM/VLB_{100} cells (Fig. 2A). Importantly, BIIB021-resitant MCF7-MDR cells showed that BIIB021 cytotoxicity was significantly enhanced by DMC or CCB treatment (Fig. 2B, Fig. 2C). Moreover, we estimated the percentage of early and late apoptotic cells in BIIB021-treated MCF7-MDR cells in the presence of absence of CCB (or DMC) cells using conventional flow cytometry (Fig. 3). In this experiment, the percentage of cells in both the lower and upper right quadrants of the Annexin/PI analysis was significantly increased by co-treatment with BIIB021 and CCB (or DMC) versus BIIB021 alone, indicating sensitization of MDR cells to BIIB021-induced apoptosis by NSAIDs. These results suggest

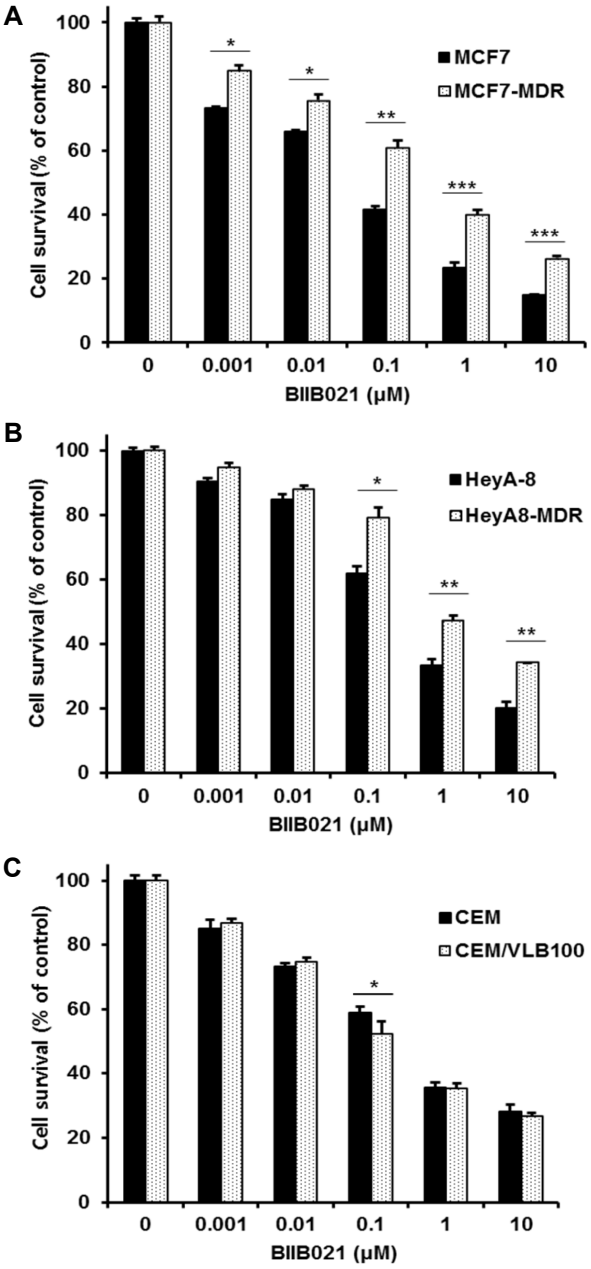

Fig. 1. Differential responses of human MDR variants to BIIB021. MCF-7, HeyA-8 and CEM cells and their MDR variants MCF7-MDR (A), HeyA8-MDR cells (B) and CEM/VLB_{100} (C) were treated with serial concentrations of BIIB021. Percentage of cell survival was determined after 96 hr of incubation using MTT assay. Results are the means ± SEs of three experiments. $*p<0.05$, $**p<0.01$ and $***p<0.001$.

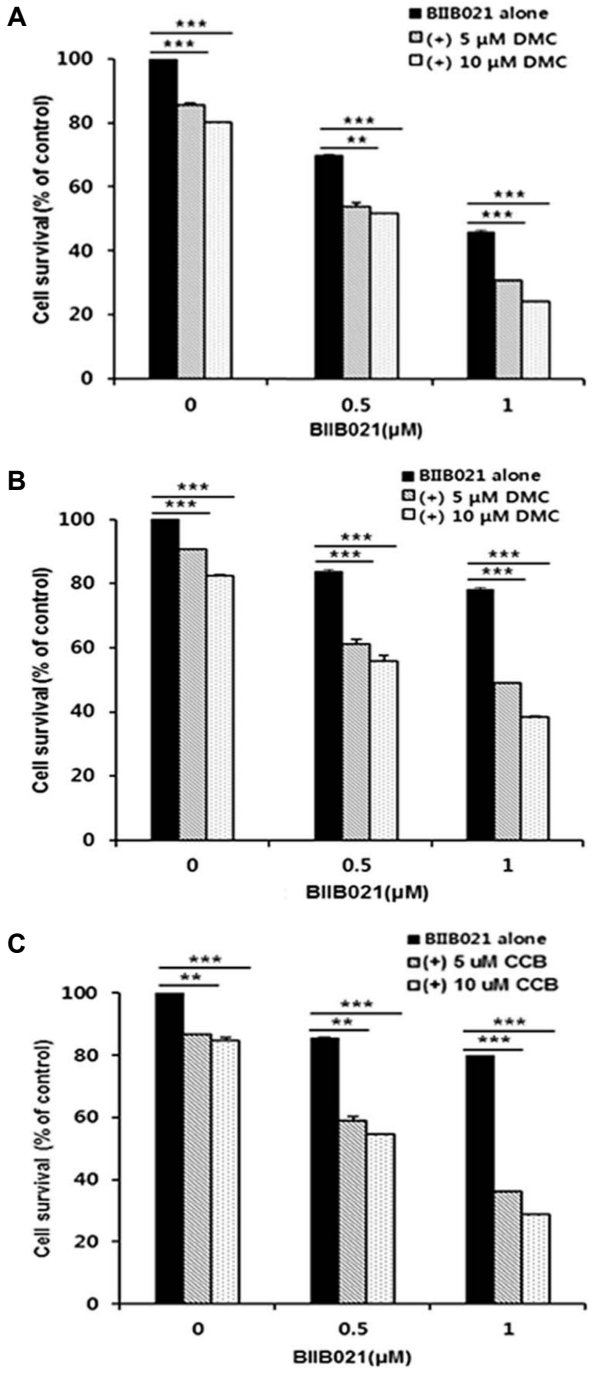

Fig. 2. Enhancement of BIIB021 cytotoxicity in MDR variants by DMC. CEM/VLB_{100} cells (A) and MCF7-MDR (B and C) were treated with serial concentrations of BIIB021 in the presence or absence of dimethyl celecoxib (DMC; 5- and 10 μM). Percentage of cell survival was determined after 96 hr of incubation using MTT assay. Results are the means ± SEs of three experiments. *p<0.05, **p<0.01 and ***p<0.001.

the possibility that NSAIDs such as DMC and CCB could be candidate sensitizers for BIIB021-resistant MDR cells.

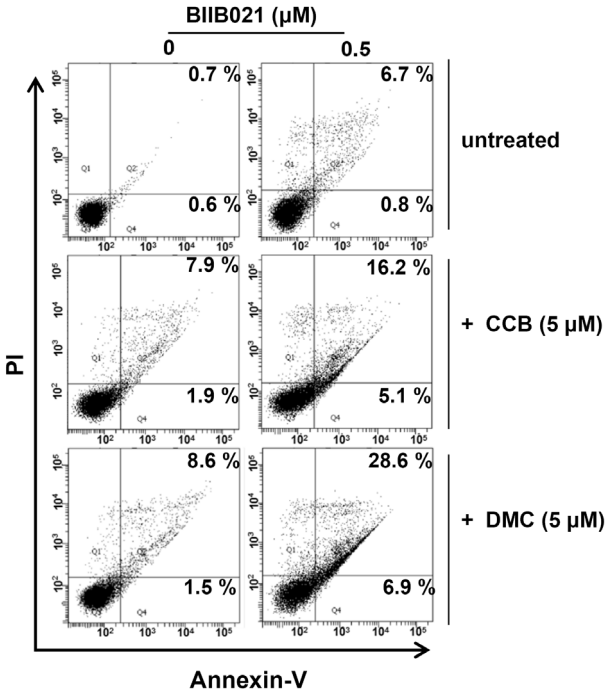

Fig. 3. Enhancement of BIIB021-induced apoptosis in MDR cells by NSAIDs. MCF7-MDR cells were treated with 0.5 μM BIIB021 and/or in the presence or absence of 5 μM CCB (or DMC) for 24 hr, and early and late apoptotic cells were analyzed by flow cytometry. Apoptosis index is defined as a percentage of early and late apoptotic cells. The lower right quadrant shows annexin positive cells (early apoptotic) and the upper right quadrant shows annexin and PI positive cells (late apoptosis cells).

Induction of autophagy and suppression of Hsp70 expression in BIIB021-treated MDR cells by NASIDs

We determined whether CCB and DMC could induce autophagic activity when CEM/VLB_{100} cells were co-treated with BIIB021 and CCB (or DMC). To measure autophagic flux in CEM/VLB_{100} cells co-treated with BIIB021 and CCB (or DMC), LC3 is the most widely used autophagosome marker because the amount of LC3-II reflects the number of autophagosomes and also degradation of p62 is another widely used marker to monitor autophagic activity because p62 directly binds to LC3 and is selectively degraded by autophagy and autophagy-related structures. Therefore, we evaluated the autophagic degradation of mutp53 in CEM/VLB_{100} cells by assessing changes in the levels of LC3-II and p62 (Fig. 4). When LC3 conversion and p62 level in CEM/VLB_{100} cells were not responded by BIIB021, CCB induced an increase of LC3 conversion and a decrease of p62 level in BIIB021-treated cells. Similarly, DMC also enhanced LC3 conversion and reduced p62 level in BIIB021-treated cells.

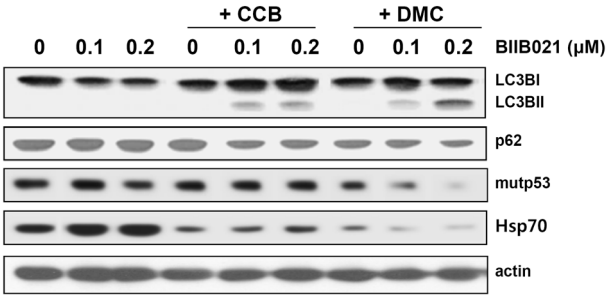

Fig. 4. Induction of autophagy and acceleration of autophagic mutp53 degradation and suppression of Hsp70 activation in MDR cells treated with BIIB021 by CCB/DMC. CEM/VLB_{100} cells were treated with BIIB021 (0.1 or 0.2 μM) in the presence or absence of 25 μM CCB (or 25 μM DMC) for 24 hr. Change levels of LC3BI/II, p62, mutp53 and Hsp70 were determined by Western blot analysis. Actin was used as a loading control.

In parallel with the autophagy-inducing effect of CCB and DMC, the level of mutp53 in BIIB021-treated cells was decreased by treatment of CCB or DMC, and importantly BIIB021-mediated induction of Hsp70 was significantly suppressed by CCB or DMC. DMC is more effective than CCB against down-regulation of mutp53 and suppression of Hsp70 induction in BIIB021-treated cells. These results indicated the possibility that combined effect of BIIB021 and CCB/DMC on the induction of autophagy would reduce BIIB021-mediated mutp53, which triggered apoptosis in CEM/VLB_{100} cells. These results suggest the possibility that NSAID including CCB and DMC could be a new class of BIIB021 sensitizer through suppression of Hsp70 induction that limits the clinical benefits of Hsp90 inhibitors.

Induction of autophagy and potentiation of 17-AAG-mediated autophagy by sulindac sulfide and niclosamide

Next, to further find new modulators of autophagy inducer for potentiation of Hsp90 inhibitor, we developed sulindac sulfide (SS) that belongs to a class of NSAIDs that has anti-tumorigenic and anti-inflammatory activities [9] and niclosamide (NC), an FDA approved oral anti-helminthic drug that has cytotoxicity in a broad spectrum of cancer cells [2]. We evaluated the autophagy inducing effect of SS and NC in MDR cells by assessing changes in the levels of LC3-II and p62. When CEM/VLB_{100} cells were treated with serial doses of SS, treatment of CEM/VLB_{100} cells with SS resulted in an increase of LC3 conversion (LC3-II) and a decrease of p62 level in a dose dependent manner, indicating autophagy-inducing ability of SS, which is associated with degradation/down-regulation of mutp53 (Fig. 5A, *left*). Similar results were observed in CEM/VLB_{100} cells treated with serial doses of NC, leading to induce autophagy as demonstrated by an increase of LC3 conversion and a decrease of p62 level, which causes degradation/down-regulation of mutp53 (Fig. 5A, *right*). We further examined whether combination of 17-AAG and SS could modulate 17-AAG-mediated-LC3-II/LC-I and p62 levels in CEM/VLB_{100} cells (Fig. 5B, *right*). SS significantly augmented 17-AAG-mediated level of LC3-II and accelerated reduction of p62 level, indicating the combined effect of 17-AAG and SS on the induction of autophagy. Moreover, the autophagy-inducing effect of SS accelerated 17-AAG-mediated down-regulation/degradation of mutp53 and c-Myc, a target gene of mutp53. Our results showed that SS-induced autophagy was associated with deg-

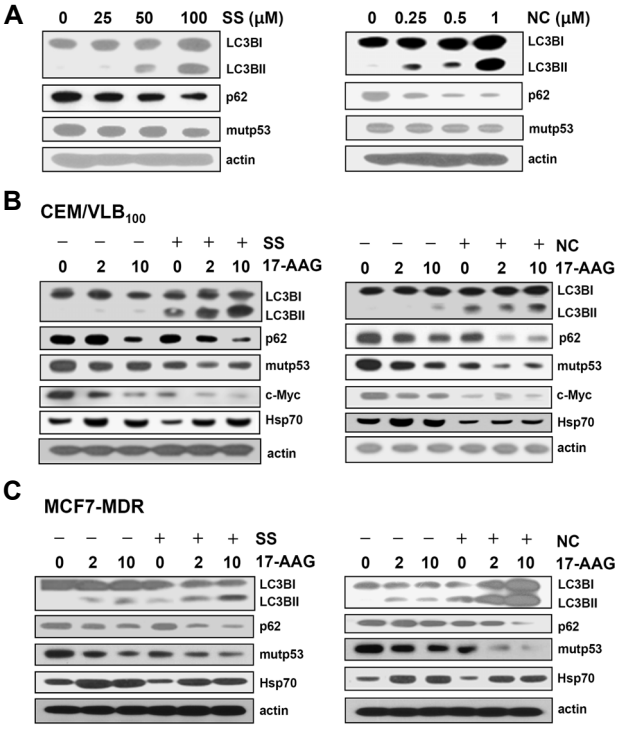

Fig. 5. Induction of autophagy and acceleration of autophagic mutp53 degradation in MDR cells treated with 17-AAG by sulindac sulfate (SS) and niclosamide (NC). CEM/VLB_{100} cells were treated with serial doses of sulindac and niclosamide for 24 hr (A), and were treated with 17-AAG (2- and 10 μM) in the presence or absence of 50 μM SS or 0.5 μM NC for 24 hr (B). MCF7-MDR cells were treated with17-AAG (2- and 10 μM) in the presence or absence of 50 μM SS or 0.5 μM NC for 24 hr (C). Change levels of LC3BI/II, p62, mutp53, Hsp70 and c-Myc were determined by western blot analysis. Actin was used as a loading control.

radation/down-regulation of c-Myc as well as mutp53 (Fig. 5B, *left*). When MCF7-MDR cells were co-treated with NC and 17-AAG, NC treatment significantly enhanced 17-AAG-mediated down-regulation/degradation of mutp53 and c-Myc via induction of autophagy (Fig. 5B, *right*). Next, we examined the effect of the presence or absence of SS (or NC) on 17-AAG-treted MCF7-MDR cells (Fig. 5C). Similarly, the autophagy-inducing effect of SS and NC accelerated 17-AAG-mediated down-regulation/degradation of mutp53 in the MDR cells. Moreover, SS and NC suppressed 17-AAG-induced Hsp70 induction, indicating possibility that reversal of Hsp90 inhibitor resistance in MCF7-MDR cells by SS and NC. Therefore, our results suggest that SS and NC could potentiate the activity of Hsp90 inhibitors via autophagy pathway.

Discussion

Hsp90 inhibitors are a group of promising antitumor agents that lead to the selective degradation of proteins involved in multiple oncogenic processes [11], and they hinder the growth of many types of tumors in both *in vitro* and *in vivo* tumor models [13]. BIIB021, a second-generation Hsp90 inhibitor, is a purine scaffold-based and a fully synthetic Hsp90 inhibitor that binds to the ATP-binding pocket of Hsp90 and interferes with Hsp90 chaperone function, which caused cell death in conjunction with alterations in expression of Hsp90 client proteins, resulting in client protein degradation and tumor growth inhibition. BIIB021 that binds selectively to Hsp90 has been evaluated in a phase I clinical trial involving solid tumors [14] and a phase II clinical trial involving gastrointestinal stromal tumors [3].

Previous study showed that BIIB021 was relatively independent of MDR1 expression and was active against P-gp expressing cell lines [19]. In this study, we found that BIIB021 cannot be applicable to all cancer cells expressing MDR proteins since MCF7-MDR and HeyA8-MDR cells were more resistant to BIIB021 than their parental counterparts but BIIB021 was sensitive to CEM/VLB_{100} cells, indicating differential responses of MDR cells to BIIB021. Moreover, we showed that CCB and its derivative DMC that belongs to the NSAIDs could be as new sensitizers of BIIB021 as well as 17-AAG in MDR cells. Indeed, NSAID-induced autophagy has diverse anticancer effects in different type of cancer cells by regulating Beclin-1, LC3-II, p62, and Atg5-12 and can modulate tumor autophagy through various signaling pathways involving PI3K/Akt/mTOR cascade [17]. Previously, we reported that autophagy inducing ability of NSAIDs involving CCB, DMC and IBU were effective for the sensitization of MDR cells to 17-AAG through inhibition of Akt/mTOR and STAT3 pathways [12]. The combined effect of BIIB021 and CCB/DMC reduce BIIB021-mediated mutp53 via the induction of autophagy, which might trigger cell death in MDR cells. In addition, our data showed that sulindac sulfide (SS) and niclosamide (NC) had autophagic autophagy-inducing ability, which may contribute to the ability of enhancing BIIB021 sensitivity. Present study showed that non-steroidal anti-inflammatory drugs (NSAIDs) such as CCB, DMC and SS induced autophagy irrespective of their COX selectivity since both DMC and SS are non-selective COX inhibitors but CCB is a cyclooxygenase (COX)-2 selective inhibitor. Indeed, COX-independent mechanisms may contribute to, or be fully responsible for their anticancer properties.

Autophagy, a self-destructive response of cells to stress, leads to degradation of endogenous cellular protein aggregates and damaged organelles by lysosomes. LC3 is converted into LC3-I, following proteolytic cleavage at the C-terminal region by autophagy-related protein-4. LC3-I is then transformed by the lipid phosphatidyl ethanolamine to LC3-II. LC3-II is deployed and attaches to the membrane of an autophagosome. LC3-II remains attached to the membrane, until the autophagosome fuses with a lysosome [6]. CCB, DMC and SS commonly enhanced LC3-II level and conversely reduced p62 level, indicating autophagy inducing ability by NSAIDs. Interestingly, we showed that an FDA-approved drug NC that is given orally to helminthosis patients also increased autophagic activity in MDR cells. It has been reported that NC is able to block the multiple signaling pathways that govern cancer initiation and progression, and also NC has potent i*n vitro* and *in vivo* anti-tumor growth activities [8]. Our study indicates the possibility that autophagy-inducing activity of NSAIDs including CCB, DMC, SS and NC could modulate Hsp90 inhibitors. We found that CCB and DMC induced an increase of LC3 conversion and a decrease of p62 level in BIIB021-treated MDR cells. Moreover, autophagy-inducing activity of DMC was associated with down-regulation of mutp53, and suppression of Hsp70 induction, which can trigger BIIB021-mediated cell death by inducing apoptosis in MDR cells. In addition, both SS and NC also enhanced 17-AAG-mediated level of LC3-II/LC-I and accelerated reduction of p62 in MDR cells.

Moreover, the autophagy-inducing effect of SS and NC accelerated 17-AAG-mediated down-regulation/degradation of mutp53 and c-Myc, a target gene of mutp53, indicating the combined effect of 17-AAG and SS/NC on the induction of autophagy and enhancement of 17-AAG cytotoxicity by SS or NC treatment.

It has been reported that BIIB021 is not susceptible to metabolism NQO1/DT-diaphorase enzymes or to efflux by P-glycoprotein, thus avoiding some of the liabilities of 17-AAG [19]. But BIIB021 up-regulated expression of the Hsp70 and Hsp27, cause of resistance to HSP90 inhibitors, in both tumor tissue and spleen [10], indicating that BIIB021 has the same mechanism of action as the natural product geldanamycin and its derivative 17-AAG as well as other Hsp90 inhibitors. Interestingly, CCB and DMC suppressed BIIB021-mediated Hsp70 induction of BIIB021-resistant MCF7-MDR cells, indicating their ability of reversing BIIB021 resistance of the cells.

In conclusion, we found potentiating effect of NSAIDs on BIIB021 activity in MDR cells, and sensitization of BIIB021-resistant MDR cells to BIIB021 through autophagic degradation of mutp53 and suppression of Hsp70 induction by NSAIDs. In addition, SS and NC possessing autophagy-inducing activity could be new sensitizers of Hsp90 inhibitors including BIIB021.

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초록 : 항암제 다제내성(MDR) 암세포의 Hsp90 저해제 BIIB021에 대한 감수성의 차이 및 NSAIDs 및 Niclosamide에 의한 Hsp90 저해제의 활성 변화

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열 충격 단백질인 heat shock protein 90 (Hsp90)은 종양 형성 과정에서 중요한 역할을 하고 있으며, 이에 따라 1세대 및 2세대 Hsp90저해제들이 개발되어, 다양한 암에서의 항암 효과가 보고되어 있다. 2세대 Hsp90저해제로 개발된 BIIB021는 1세대 Hsp90저해제인 17-allylamino-17-demethoxygeldanamycin (17-AAG)에 내성을 나타내는 항암제 다제내성(MDR) 암세포에 감수성을 가진다고 알려져 있지만, 본 연구에서 BIIB021에 내성인 MDR세포로서, MCF7-MDR 및 HeyA8-MDR세포가 해당됨을 밝혔다. BIIB021 감수성을 증강시키는 물질로 비스테로이드성 항염증약물(NSAID)인 dimethyl-celecoxib (DMC)의 BIIB021의 효과 증강 활성을 BIIB021-내성 및 -감수성 MDR 세포에서 확인하였다. MDR세포에 NSAID와 BIIB021의 병합 처리한 경우, NSAID의 자가분해(autophagy) 유도 활성에 의해 MDR세포에서 과잉 발현하는 변이형 mutant p53 (mutp53)을 분해할 뿐만 아니라 BIIB021 처리로 유도되는 Hsp70 발현을 억제하므로써, 암세포의 BIIB021 내성을 극복할 수 있는 활성을 나타내었다. 또한 NSAID 물질인 sulindac sulfate 및 FDA 승인 약물인 niclosamide 도 자가분해 유도 활성으로 Hsp90의 타켓 단백질 인 mutp53 및 c-Myc의 분해를 유도하므로서, 17-AAG 효과를 증강시켰다. 그러므로 본 연구에서는 새로운 BIIB021에 대한 효과 증강 및 내성 극복 물질로서, NSAIDs 및 niclosamide를 발굴하였으며, 이들 물질의 자가분해 경로 활성화에 의하여, BIIB021 효과를 극대화 시킴을 밝혔다.