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Short Communication

Trafficking Inhibition of Bruceanol B as a Radical-Producing Antibiotic

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Bruceanol B, isolated from a soil microbe strain DS4, generated oxygen radicals in *Bacillus subtilis* lysates. Bruceanol B inhibited the trafficking of viral glycoprotein in virus-infected baby hamster kidney (BHK) cells. Bruceanol B also effectively inhibited syncytium formation in a dose-dependent manner. However, glycoprotein synthesis was not affected by the compound. Results indicate that bruceanol B generating oxygen radical has inhibitory activity in the trafficking of viral glycoprotein of BHK cell.

Key words: bruceanol B, radical-generating antibiotic, trafficking inhibition

In performing high-throughput screening for the radical-generating antibiotic, bruceanol B was isolated from a soil microorganism (Fig. 1). This compound is known as anti-cancer compound [Okano *et al*., 1985]. Here, the results of *in vitro* studies on the relationship between antibiotic generating radicals and trafficking inhibition in cells is reported. Antibacterial activity of bruceanol B against mutant or wild-type *Bacillus subtilis* was assessed according to the zone of growth inhibition [Bauer *et al*., 1966]. *B. subtilis* was inoculated into sterile Mueller-Hinton agar (Becton Dickinson, Sparks, MD) for agar diffusion assay. Forty or fifty microliters of samples, dissolved in Dimethyl sulfoxide or methanol, were loaded on an aseptic paper disc (6 mm diameter, Advantec. Tokyo, Japan). The solvents were allowed to evaporate in a stream of sterile air before disc deposition on the surface of an agar medium. After pre-diffusion of the compounds into the agar in a refrigerator, the plates were incubated for 24 h at 37°C. Bruceanol B displayed antibacterial activity in a dose-dependent manner (Fig. 2A). Moreover, bruceanol B inhibited the growth of the rec- strain (*B. subtilis* KCTC 3069, ○) more potently than that of the rec+ strain (*B. subtilis* ATCC6633, ●). IC_{50} value (25 μg/mL) of this compound against rec- strain is lower than that (50 μg/mL) against rec+ strain (data not shown). DNA strand scission is known to be caused by the active oxygen radical [Suzuki *et al*., 1983], and recombinant-deficient mutants generally show a higher sensitivity to antibiotics, which cause DNA damage [Love *et al*., 1985; Osburne *et al*., 1993]. Antibacterial activities of reactive oxygen species are prevented by scavenging agents. Accordingly, the effects of dithiothreitol (DTT) on the antibacterial activity of bruceanol B were examined with *B. subtilis* KCTC 3069, which is vulnerable to oxygen stress. Bruceanol B displayed antibacterial activity against *B. subtilis*, which was reduced upon the addition of 250 μM DTT (data not shown). To confirm the generation of superoxide radicals, lysates prepared from *B. subtilis* were treated with bruceanol B, and the superoxide radicals generated were measured by the nitroblue tetrazolium (NBT) reduction method [Nomoto *et al*., 1988; Lee *et al*., 1999]. *B. subtilis* KCTC 3069 (rec-) was homogenized in 0.1 M potassium phosphate buffer (pH 7.0) containing 150 mM KCl and 1 mM EDTA, and the supernatant after centrifugation at 5,000 rpm for 20 min was used for the assay. The reaction mixture (1 mL) including the supernatant (0.8 mg protein/mL), 0.05% NBT (Sigma, St. Louis, MO), and various concentrations of the bruceanol B was incubated

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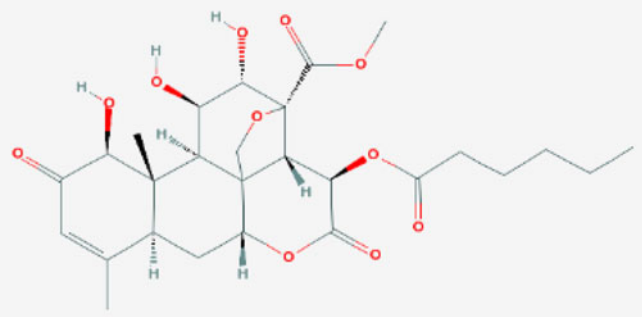

Fig. 1. Structure of bruceanol B.

for 30 min at 37°C in the presence (open square, □) or absence (closed square, ■) of 130 μg/mL superoxide dismutase (SOD). The reaction was stopped by the addition of 2 mL of 1 N HCl. The whole mixture was centrifuged at 5,000 rpm for 20 min, and precipitates were washed with 1 N HCl, dissolved in 1 mL of hot pyridine (Sigma), and the absorbance at 515 nm was measured using a spectrophotometer. Bruceanol B generated superoxide radicals in a dose-dependent manner (Fig. 2B). Superoxide radicals, however, were strongly detoxified by the addition of SOD (130 μg/mL). These results indicate that the antibacterial activities of bruceanol B in *B. subtilis* resulted from the generation of reactive oxygen species by the regents. Recently, radical-producing substances were shown to inhibited trafficking processes of glycoproteins in baby hamster kidney (BHK) cells [Lee *et al*., 2010]. Trafficking is achieved by repeated cycles of budding and the fusion of transport vesicles [Wattenberg, 1990; Hiebsch *et al*., 1992; Bos *et al*., 1995]. Significant efforts have been made to determine the mechanism(s) underlying glycosylation trafficking in cells, particularly with the aid of inhibitors of trafficking,

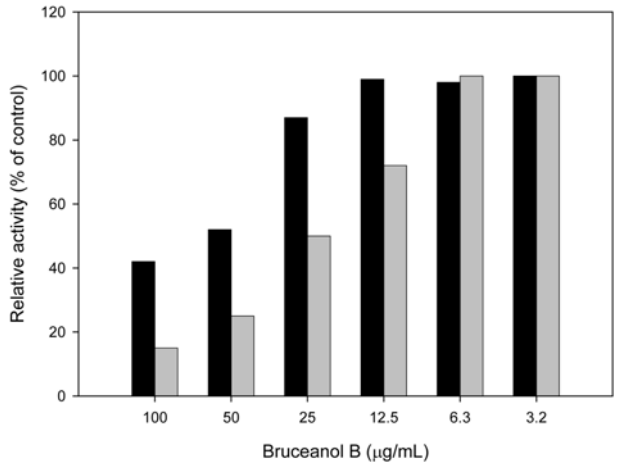

Fig. 3. Blockade of the cell surface expression of NDV-HN glycoprotein by bruceanol B. Monolayer cultures of BHK cells in 6-well or microtiter plates were infected with NDV, and indicated concentrations of bruceanol B were added to the cultures 1 h after infection. Percentages of HAU (black) or HAD (gray) were determined at 14 h of infection. NDV-HN protein synthesis was quantified by determining HAU in whole lysates of infected cultures in microtiter plates, and cell surface expression quantified by measuring the amounts of chicken red blood cells adsorbed to intact infected cells in 6-well plates. Results are expressed as a percentage of the control value.

which are powerful tools in these studies [Nakamura *et al*., 2003]. However, limited compounds that affect intracellular trafficking processes have been identified to date. In preliminary data [Lee *et al*., 2010], we hypothesize that radical-producing antibiotics partially act as trafficking inhibitors of glycoprotein in cells. To establish whether bruceanol B, as a radical-producing agent, inhibits the trafficking of glycoprotein, its effects on the cell surface

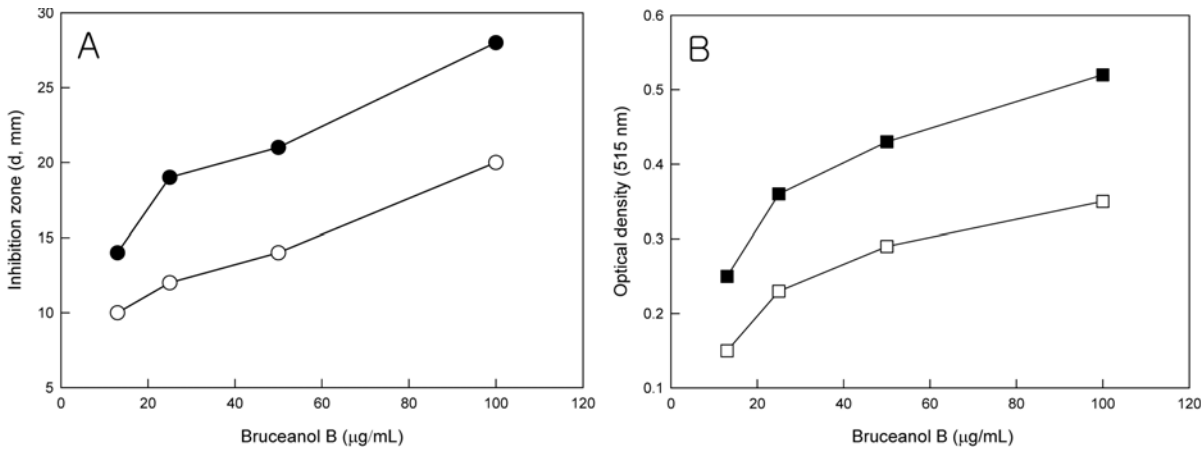

Fig. 2. Antibacterial activities of bruceanol B against wild-type (rec+, ●) and mutant (rec-, ○) strains of *B. subtilis* (A) and superoxide radical generation in *B. subtilis* KCTC3069 cell lysates by bruceanol B in the presence (open square, □) or absence (closed square, ■) of 130 μg/mL superoxide dismutase (SOD) (B). Activity was expressed as the diameter of growth inhibition zone surrounding paper discs on the assay plate (see Text).

expression of viral glycoproteins in BHK cells were examined. The physiological changes of newcastle disease virus (NDV) in BHK cells are similar to those of the human immunodeficiency virus (HIV). HIV infection *in vitro* induces syncytium formation by cell-to-cell fusion [Quinn, 2008]. To establish whether bruceanol B inhibits syncytium formation without significantly affecting glycoprotein synthesis, its effects on the cell surface expression of viral glycoproteins were examined. BHK cells in 6-well plates were infected with NDV and incubated at 37°C for 12 h in the presence of bruceanol B, as a radical-producing agent, at the concentrations indicated in Fig. 3. Synthesis of NDV-hemagglutinin-neuramidase (HN) glycoprotein was quantified by determining hemagglutination units (HAU) in whole lysates of NDV-infected cells. NDV-HN expressed on the cell surface was quantified by hemadsorption (HAD), as described previously [Lee *et al*., 2010]. To quantify the total amount of NDV-HN synthesized, whole NDV-infected cultures were disrupted by brief sonication, and chicken red blood cells were added to determine hemagglutination activities in lysates. HAU was not significantly decreased at concentrations up to 25 μg/mL bruceanol B (Fig. 2, black). However, the binding of extracellularly added chicken red blood cells to the surfaces of intact NDV-infected cells (expressed as % HAD) proportionally decreased to the bruceanol B concentration (Fig. 2, gray). These results suggest that the compound hinders glycoprotein expression on the cell surface through inhibition of trafficking process rather than glycoprotein synthesis. The potential of trafficking inhibitors as radical-generating antibiotic requires further investigation as a candidate of anti-HIV therapeutic agents.

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