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Mechanisms of beauvericin toxicity and antioxidant cellular defense

Beatriz Mallebrera*, Ana Juan-Garcia, Guillermina Font, Maria-Jose Ruiz

Laboratory of Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andres Estelles s/n, 46100 Burjassot, Valencia, Spain

HIGHLIGHTS

- BEA produces cytotoxic effects in CHO-K1 cells.
- BEA alters mitochondrial membrane potential in CHO-K1 cells.
- BEA produces DNA strand breakage in CHO-K1 cells.
- At 24 h, BEA exposure arrests G_0/G_1 phase of cell cycle and produces apoptosis.
- Enzymatic defense (SOD and CAT) increases in CHO-K1 cells exposed to BEA.

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ABSTRACT

Beauvericin (BEA) is a secondary metabolite produced by many species of fungus *Fusarium*. This study determines the injury (cell viability, cell proliferation, mitochondrial membrane potential, cell death and DNA damage) and the intracellular defense mechanisms (catalase and superoxide dismutase) in Chinese Hamster ovary (CHO-K1) cells after BEA exposure. The results obtained in this study demonstrated that BEA induces cytotoxicity in a dose- and time-dependent manner in CHO-K1 cells. Moreover, disruption in mitochondrial enzymatic activity and cell proliferation has been observed after BEA exposure, which can lead or be consequence of cell death. BEA inhibits cell proliferation by arresting cells in G_0/G_1 and increasing apoptosis. Moreover, at higher exposure times, BEA induces differentiation of CHO-K1 cells through G_2/M arrest, preventing that cells entry into mitosis. DNA strand breaks were observed at 1 μM after 24 h of exposure. On the other hand, the SOD and CAT activities were increased after BEA exposure and as a defense system they could contribute to eliminate damage produced by BEA and oxidants products generated in CHO-K1 cells.

1. Introduction

Mycotoxins are secondary metabolites produced by fungi. They can damage health in humans and animals through ingestion, inhalation or the skin contact (Marin et al., 2013). Emerging *Fusarium* mycotoxins include fusaproliferin, enniatins, beauvericin and moniliformin.

Beauvericin (BEA) is synthesized by many species of fungus *Fusarium* and *Beauveria bassiana*. Wheat, rice, corn, barley and cereal derivate are known as susceptible commodities to be contaminated by BEA (Meca et al., 2010; Mahnine et al., 2011; Zinedine et al., 2011). BEA has ionophoric activity, it is incorporate into biological membranes forming a complex with essential cations, which increases its ion permeability and affects cellular homeostasis (Kouri et al., 2003; Tonshin et al., 2010). Therefore, the mitochondria membrane may be one of the possible sites for BEA-mediated cytotoxicity. Reactive oxygen species (ROS) production in excess of the capabilities of detoxication systems causes oxidative stress in the cell. ROS are extremely reactive, making them likely to participate in chemical reactions. These reactions also damage lipids, proteins and DNA and cause deleterious effects (Kanduc et al., 2002). Oxidative reactions in mitochondria could induce its dysfunction which activates the apoptotic and necrotic pathways (Tonshin et al., 2010; Prosperini et al., 2013a). ROS can also damage DNA causing single-strand breaks and base alteration inducing DNA adducts and mutation.

Because of the propensity of ROS to react with and damage important bimolecules, cells have evolved biochemical systems for detoxication. Antioxidant compounds (such as glutathione, and vitamins and polyphenols ingested in diet) and enzymes (glutathione peroxidase, GPx; catalase, CAT and superoxide

* Corresponding author.
E-mail address: beatriz.mallebrera@uv.es (B. Mallebrera).

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dismutase, SOD) contribute to protect the cells from oxidative stress and help maintain the redox balance (Matés, 2000).

Stress response reflects an imbalance between pro-oxidant activities of substances and the innate cytoprotective response of the cells. Mycotoxins can disturb this balance either by increasing the pro-oxidant activity or by disrupting the protective pathways. The aims of this study were to determine the injury of BEA in biological CHO-K1 cell components after 24, 48 and 72 h of exposure. And the enzymatic antioxidants defense mechanisms in CHO-K1 cells after BEA exposure.

2. Material and methods

2.1. Reagents

The reagent grade chemicals, cell culture components used and BEA (783.95 g/mol, ≥97% purity) were purchased by Sigma chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) was from Cambrex Company (Belgium). Stock solution of BEA was prepared in methanol and maintained at −20 °C. Final concentrations of BEA in the assay were achieved by their dilution in the culture medium. The final methanol concentration in the medium was ≤1% (v/v).

2.2. Cell culture

CHO-K1 cells derived from Chinese Hamster ovary, were grown in 9 cm^2 polystyrene tissue culture dishes in Ham's-F12 medium supplemented with 25 mM HEPES buffer, 10% (v/v) FBS, 100 U/mL penicillin and 100 mg/mL streptomycin. Incubation conditions were pH 7.4, 37 °C under 5% CO_2 and 95% air atmosphere at constant humidity. Absence of mycoplasma was checked routinely using the mycoplasma stain kit (Sigma–Aldrich, St. Louis, MO, USA).

2.3. Cell viability assay

CHO-K1 cells were cultured in 96-wells microplates at a density of 2×10^4 cells/well. Counting of cells was performer with a Beckman coulter (Florida, USA). The cells were cultured until confluence (65%). Then, the culture medium was removed and replaced with fresh medium containing different concentrations of BEA (0.625, 1.25, 2.5, 5, 10 and 20 μM). Cytotoxicity was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assays as described by Ruiz et al. (2006) during 24, 48 and 72 h. The MTT assay monitors a reduction of yellow tetrazolium salt by mitochondrial dehydrogenase enzymes of metabolically activate/viable cells to purple formazan crystals. Absorbance was measured at 570 nm with an automatic ELISA reader. The IC_{50} values obtained were calculated from full dose–response curves. Three assays in different days were performed.

2.4. Cell proliferation

Cell proliferation is essential for the homeostasis of most organs and tissues, BEA can interfere with this process. The key to this effect is the cell cycle, which is closely linked to apoptosis. Cell cycle analysis was performed by staining the DNA with propidium iodide (PI) as described previously (Juan-García et al., 2013). 6.8×10^5 cells/well were seeded in 6-well plates and treated with BEA at 0.1, 1 and 5 μM for 24, 48 and 72 h. Then the cells were trypsinized and place on ice for 30 min with 860 μL of fresh medium containing 29 ng/mL of Vindelov's PI staining solution prepared as follows: 10 mM Tris base, 50 μg/mL of PI, 0.1% Triton X-100, 10 mg (700 U/L) RNase A (Sigma–Aldrich) and 10 mM NaCl. Four independent experiments were performed for each BEA treatment and at least 10,000 cells were analyzed for each sample at the excitation and emission wavelengths of 488 and 620 nm, respectively.

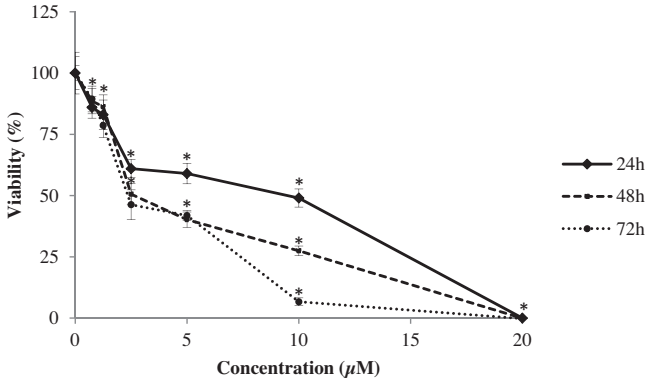

Fig. 1. Cell viability (%) in CHO-K1 cells exposed to BEA by MTT assay after 24, 48 and 72 h. Data are expressed as mean ± SEM ($n = 3$). $^*p \leq 0.05$ indicates significant differences from the control.

2.5. Mitochondrial membrane potential ($\Delta\Psi m$)

Mitochondrial membrane potential ($\Delta\Psi m$) was measured using a modified rhodamine 123 (Rh123) method described by Andersson et al. (1987). CHO-K1 cells were cultured in 96-wells black microplates at a density of 2×10^4 cells/well. After confluence cells were exposed to BEA (0.1, 1 and 5 μM) for 24 h, and then were incubated with Rh123 at final concentration of 5 μM for 15 min in darkness. The Rh123 was removed and the cells were resuspended in PBS. The fluorescence of the cationic dye Rh123 was measured at emission and excitation wavelengths of 485 and 530 nm, respectively. Three replicates were performed. The results are expressed as percentage (%) of the control.

2.6. Apoptosis and necrosis determination

Cell death generally proceeds through two molecular mechanisms: necrosis and apoptosis. One of the characteristics of apoptosis is the externalization of phosphatidylserine (PS) to the outer leaflet of the plasma membrane. The differential of population of apoptotic cells (early or late), necrotic and dead cells was identified by fluorescein isothiocyanate (FITC)-labeled Annexin V (Annexin V-FITC) and PI double staining (Vermes et al., 1995). A total of 6.8×10^5 cells/well were seeded in 6-well plates. After 24, 48 and 72 h of exposure at 0.1, 1 and 5 μM BEA, the assay was carried out as described by Juan-García et al. (2013). The cells were resuspended in 400 μL Annexin-V-binding buffer. Then, they were situate on ice and darkness 30 min with 1.25 ng/mL Annexin V-FITC and 10 μg/mL PI dyes to link the PS in the presence of Ca^{2+}. Positioning of quadrants on Annexin V-FITC/PI dot plots was performed. Pro-apoptotic/apoptotic (early apoptosis) cells (Annexin V-FITC+/PI−), in Annexin V-FITC+/PI+ cells have completed the apoptotic process and start the necrotic process (referred as apoptotic/necrotic or late apoptosis) or are already dead, necrotic cells (Annexin V-FITC−/PI+) and living cells (Annexin V-FITC−/PI−). 10,000 cells were acquired and analyzed on a BD FACS Canto flow cytometer with FACSDiva software v 6.1.3 (BD Biosciences). Green (FL-1530 nm) and orange–red fluorescence (FL-2585 nm) were detected, emitted by FITC and PI, respectively. Quadrant statistics were performed to determine viable cells, early apoptotic, late apoptotic and dead cells from the total population of cells. Determinations were performed in four independent experiments.