Sensitivity of Hep G2 Cells to Bacillus cereus Emetic Toxin

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ABSTRACT. We herein examined the sensitivity of Hep G2 human hepatoma cells to Bacillus cereus emetic toxin. Hep G2 cells were treated with the emetic toxin, and the cell shape was observed. The same experiments were performed for comparison purposes, using HEp-2 cells, which are currently used by most laboratories for a bioassay of the emetic toxin. Hep G2 cells showed clearer vacuolation in the cytosol within 2 hr and required a shorter incubation period than HEp-2 cells (10 hr). The number of vacuoles in the Hep G2 cells was greater, and the size of the vacuoles was larger than those observed in HEp-2 cells. The minimal concentration of the emetic toxin required to induce the vacuolation of Hep G2 cells was 0.04 ng/ml. The concentration for the HEp-2 cells was 1 ng/ml. These findings indicate that Hep G2 cells show higher sensitivity to the emetic toxin. Hep G2 cells may be superior to the currently used HEp-2 cells for the bioassay of the emetic toxin.

KEY WORDS: Bacillus cereus, emetic toxin, hep G2 cell, vacuolation.


Bacillus cereus food poisoning is classified into two types; one presents diarrheal, and the other emetic syndromes. Diarrheal syndrome in B. cereus food poisoning is caused by enterotoxin, and vomiting is triggered by an emetic toxin [16]. Several fatal cases of the emetic type of food poisoning have been reported in Switzerland (1997, 2009, 2011), Japan (2009, 2011, and Belgium (2011, [13]). The emetic toxin is a dodecapepsipeptide, with a molecular weight of 1153 [1]. The emetic toxin is a circular peptide and is heat stable, i.e. resistant to cooking.

HEP-2 is a human cell line derived from a larynx carcinoma [12]. Hughes et al. reported that the emetic toxin caused vacuolation in the cytosol of HEP-2 cells [8]. Agata et al. isolated and purified the emetic toxin, using the vacuolation phenomenon in the HEP-2 cells as a marker indicating the active toxic fractions [1]. Because of the ease of cell culture, the use of the vacuolation assay employing HEP-2 cells has become wide-spread [2, 14, 18]. Although a color-developing assay monitoring the cell-death ratio has been proposed, non-specific cell-death was noted due to mitochondrial damages, when using this assay [6]. However, the HEP-2 cell assay also has many disadvantages. For instance, long-term personal experience is needed to recognize the vacuoles in the cytosol. Vacuolation does not occur in all of the cells treated, even those treated with higher concentration of the emetic toxin: only 10 to 30% of the cells show vacuolation. These characteristics of the HEP-2 cell assay suggest that the protocol requires improvement to be clearer, to provide more rapid results, and to be more convenient. A direct cause of the fatal cases of B. cereus food poisoning was liver failure [11, 13, 17]. This finding indicated that the emetic toxin is hepatotoxie. The Hep G2 cell line is of human hepatoma origin [5]. We hypothesized that these liver cells would be useful for detecting the emetic toxin, and herein examined the sensitivity of Hep G2 cells to the emetic toxin.

The emetic toxin was purchased from Biocontrol Inc. (Nagoya, Japan), and the manufacturer indicated that the titer of the emetic toxin required to induce the vacuolation in HEP-2 cells was 1 ng/ml. The minimal effective concentration of the emetic toxin to induce vacuoles in HEP-2 cells was 0.5 to 2.0 ng/ml under our experimental conditions. The HEP-2 cells used in our study were kindly provided by Dr. Shigeko Ueda (Kagawa Nutrition University, Saitama, Japan). Hep G2 cells were purchased from the Human Science Foundation (Tokyo, Japan). HEP-2 cells were maintained in Basal Medium Eagle (BME, Sigma, St.Louis, MO, U.S.A.) containing 10% fetal bovine serum (FBS, Valley Biomedical, Winchester, VA, U.S.A.) and non-essential amino acids (Sigma). Hep G2 cells were maintained in Dulbecco’s modified Eagle MEM (DMEM, Sigma) containing 10% FBS. Both cell lines were seeded into 25-cm² flasks at 1 × 10⁶ cells/flask, and then were incubated in a CO₂-incubator. The cells were used 2 days after seeding.

The bioassays using HEP-2 cells or Hep G2 cells were performed according to the previously reported method [1, 18]. Briefly, 25 µl of BME or DMEM was placed in every well of 96-well culture plates. A 1-µl aliquot of the emetic toxin (1 mg/ml in 75% methanol containing 250 µM KCl) was added into the first well containing 49 µl of the medium, and the solutions were mixed. Thereafter, 25 µl of the mixture was transferred to the neighboring well, mixed,
Y. KAMATA ET AL.

and transferred repeatedly in the same way. The cells were harvested daily after seeding, the cellular concentration was counted, and then the cells were used for a bioassay to evaluate the sensitivity of the cells to the emetic toxin. Freshly suspended Hep G2 or HEp-2 cells (1 × 10⁵ cells/ml) in BME or DMEM containing 1% FBS were added into wells at 100 µl/well. The plates were then incubated in a CO₂ incubator at 37°C. The cells were photographed 24 or 48 hr after toxin treatment. More than 100 cells were counted, and their vacuoles were identified on the photographs.

The HEp-2 and Hep G2 cells treated with the emetic toxin were observed under a Cell Observation Module (Model MCOK-F110-AS, Sanyo, Osaka, Japan), which has a fixed microscopic view. The module was placed in a CO₂ incubator, and was controlled with the software program provided by the manufacturer.

The emetic toxin was added into the 35-mm culture dishes containing attached HEp-2 and Hep G2 cells. Next, the shape of both cell lines was recorded every 15 min at a fixed angle. Small and unclear vacuoles appeared in the cytosol of HEp-2 cells 5 hr after toxin treatment. The vacuoles became clearer and typical after a 10-hr treatment period (Fig. 1). On the other hand, the shape of the Hep G2 cells changed more quickly. Clear and distinctive vacuoles were found only 2 hr after addition of the toxin. The vacuoles in the Hep G2 cells were larger, and the number of vacuoles was increased in comparison to those of the HEp-2 cell. Only 2 to 5% of the HEp-2 cells were vacuolated after 12 hr of treatment, while 25 to 60% of the Hep G2 cells were vacuolated after 12 hr. After a 24-hr incubation, the vacuolation rate of the HEp-2 cells was 20 to 30%, and that of the Hep G2 was 60% or more. The number of vacuole-bearing cells in the well containing Hep G2 cells was also higher than that in the HEp-2 cells 48 hr after toxin treatment (Fig. 1, panels A and B). Therefore, at all time points examined, the number of vacuoles in the Hep G2 cells was higher, and the vacuoles

![Fig. 1](image_url)
were larger and more clearly distinguishable than those of Hep-2 cells.

The minimal concentration of the emetic toxin required to induce vacuoles in the Hep-2 cells prepared 2 days after seeding ranged from 0.5 to 2 ng/ml. The minimal effective concentration for Hep G2 cells was examined every day after the seeding of the cells. The concentrations were changed, depending on the cell number (Table 1). On day 2 after seeding, the cell concentration reached 2 × 10⁶ cells/flask, and a small amount of the toxin was observed to induce vacuoles in the Hep G-2 cells: namely, a concentration range from 0.01 to 0.08 ng/ml with a median of 0.04 ng/ml, was able to induce vacuoles formation. On days 3 and 4, the cells proliferated, and the minimal effective concentrations were found to be higher than those on day 2.

There are several methods that can be used to detect and quantify the concentration of emetic toxin, such as LC/MS [4, 7], bore sperm [3, 9, 15], isolated mitochondria [10], and cultured cells [2, 14, 18]. Each method has advantages and disadvantages; cost, required skill level of the technician, maintenance, difficulty to perform, time required for results, etc. For example, LC/MS instruments are expensive, rats have to be sacrificed to prepare isolated mitochondria, and bore sperms are generally unfamiliar to inspectors working in the laboratories examining food poisoning. Due to the ease of performing, and to the low cost, bioassays using cultured cells should be considered. The Hep G2 cells were found to show useful properties to detect and quantify the emetic toxin. The higher sensitivity of Hep G2 cells to the emetic toxin might contribute to the development of a new bioassay. Furthermore, because the Hep G2 cell line was established from human hepatoma, these cells might be useful to study liver failure resulting from exposure to the emetic toxin.

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REFERENCES