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Induction of calcium influx from extracellular fluid by beauvericin in human leukemia cells ☆

Bing-Fang Chen, Meng-Chao Tsai, Guey-Mei Jow *

School of Medicine, Fu Jen Catholic University, 510 Chung-Cheng Rd., Hsin-Chuang, Taipei, Hsien 24205, Taiwan

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Abstract

Beauvericin, a cyclic hexadepsipeptide, is a mycotoxin that can induce cell death in human lymphoblastic leukemia CCRF-CEM cells. Our previous data have shown that beauvericin induces cell death in CCRF-CEM cells in a dose- and time-dependent manner, and that this beauvericin-induced cell death can be prevented by administration of intracellular calcium chelator-BAPTA. Therefore, the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) may play an important role in beauvericin-induced cell death in CCRF-CEM cells. In this study, the effect of beauvericin on $[Ca^{2+}]_i$ and the possible mechanism responsible for the changes of $[Ca^{2+}]_i$ in CCRF-CEM cells were investigated. Beauvericin caused a rapid and sustained $[Ca^{2+}]_i$ rise in a dose-dependent manner. Excess extracellular Ca^{2+} facilitated beauvericin-induced $[Ca^{2+}]_i$ rise by adding 1 mM $CaCl_2$ in the bathing medium. On the other hand, beauvericin-induced $[Ca^{2+}]_i$ rise was prevented in Ca^{2+}-free Tyrode's solution by 200 μM EGTA. In addition, beauvericin-induced $[Ca^{2+}]_i$ rise was also attenuated by intracellular Ca^{2+} chelator-BAPTA/AM. It is worthy to note that neither the voltage-dependent Ca^{2+} channel blocker, nimodipine, nor depletion of intracellular Ca^{2+} with thapsigargin, an endoplasmic reticulum Ca^{2+} pump inhibitor, has any effect on beauvericin-induced $[Ca^{2+}]_i$ rise. The data from present study indicate that beauvericin acts as a potent Ca^{2+}mobilizer by stimulating extracellular Ca^{2+} influx CCRF-CEM cells.

Keywords: Beauvericin; Fluo-3; Intracellular calcium; Apoptosis; CCRF-CEM cells

Beauvericin, a cyclic hexadepsipeptide, is extracted from the insect-body portion of chan-hua (fungus: *Codyceps cicadae*) [1]. Beauvericin is also produced by several phytopathogenic *Fusarium* species, parasitic to maize, wheat, rice, and other important commodities [2–7]. Chan-hua has long been used in traditional Chinese medical practice for the treatment of childhood convulsion, palpitation, and sedation. It has been shown that beauvericin can modulate intracellular electrolyte concentrations and cellular functions. Beauvericin inhibited the L-type Ca^{2+} current in the NG108-15 neuronal cell line and increased the intracellular calcium by increasing the formation of cation-selective channels in lipid membrane [8,9]. Our previous studies have shown that beauvericin-induced cell death can be prevented by administration of intracellular calcium chelator-BAPTA/AM [10] in human lymphoblastic leukemia CCRF-CEM cells, indicating that the intracellular Ca^{2+} plays an important role in cell death signaling.

The process of apoptosis is regulated by cell surface signals and expression of specific intracellular proteins [11,12]. It has been suggested that apoptosis is associated with calcium signaling in some cell type [13,14]. Intracellular Ca^{2+} seems to be an important component of the mechanisms of apoptosis. Calcium signaling is upstream of certain pathways that lead to apoptosis [15]. A variety of toxic insults associated with increasing intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) can result in endoplasmic reticulum stress, morphological alterations of cells, and ultimately cell death

☆ *Abbreviations:* BEA, beauvericin; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

* Corresponding author. Fax: +886 2 2905 2096.
E-mail address: nurs1019@mails.fju.edu.tw (G.-M. Jow).

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[13,14,16]. Elevation of cytosol (intracellular) calcium concentration has been demonstrated to cause proapoptotic protein expression [14,17]. In some mammalian cells, cell death caused by beauvericin has been suggested to be the involvement of a Ca^{2+}-dependent pathway, in which beauvericin induced a significant increase in $[Ca^{2+}]_i$ that leads to a combination of cellular apoptosis and necrosis responses [10,18,19]. The mechanism(s) underlying beauvericin-induced $[Ca^{2+}]_i$ increase, whether Ca^{2+} from Ca^{2+} influx of intracellular or extracellular stores, remains inconclusive. Beauvericin induces apoptotic changes such as DNA fragmentation which has been demonstrated to take place in the complete absence of extracellular Ca^{2+}, suggesting that beauvericin triggers release of calcium from endoplasmic reticulum [18]. It has also been demonstrated that beauvericin drives cyotosolic calcium increase from intracellular storage and proceeds to cytotoxicity in human macrophage cell line THP-1 [19]. Using the BAPTA/AM, an intracellular calcium chelator, the beauvericin-induced cell death was diminished [10]. However, our recent studies demonstrated that extracellular Ca^{2+} influx contributes, at least partially, to the mechanism of beauvericin-induced cell death in *Xenopus* oocytes [20]. The physiochemical studies have proposed beauvericin to be considered a potential ionophore permeable to or capable of transporting cations such as K^+, Na^+, and Ca^{2+} [9,21–23]. In addition, "sandwiching" of beauvericin (Fig. 1) molecules during cation complexation was predicted. The majority of the evidence for ionophoric properties of beauvericin can be explained by a carrier and/or a channel-forming mechanism. However, the direct evidence of beauvericin affecting extracellular Ca^{2+} influx has not yet been demonstrated.

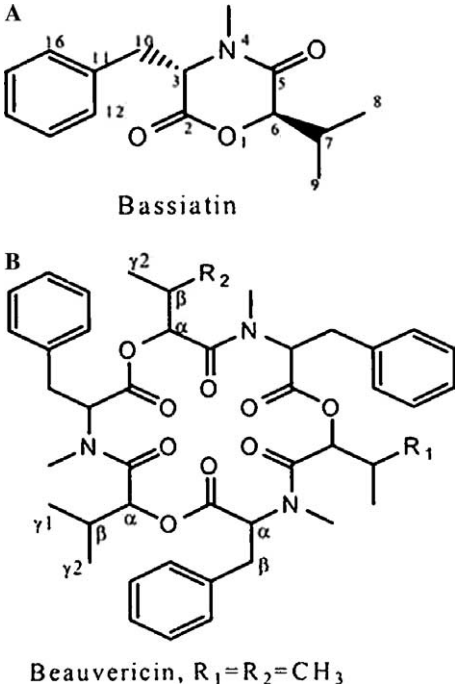

Fig. 1. Chemical structures of bassiatin (A) and beauvericin (BEA, B).

In this study, using Fluo-3 as fluorescent Ca^{2+} probe, we have demonstrated, for the first time, that beauvericin caused a significant raise of $[Ca^{2+}]_i$ in a dose-dependent manner in CCRF-CEM leukemia cells. CCRF-CEM cells were chosen because previous studies suggested that BAPTA, an intracellular Ca^{2+} chelator, prevents beauvericin-induced cell cytotoxicity in this cell line [10].

Materials and methods

Cell culture. Human lymphoblastic leukemia CCRF-CEM cells were maintained in RPMI-1640 medium (Hyclone) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone), 100 μg/ml penicillin, 100 μg/ml streptomycin, and 100 μg/ml amphotericin B (Hyclone). The cells were grown in a humidified incubator at 37 °C under a 5% CO_2/95% air atmosphere. For each experiment, 3×10^5 cells were seeded in each well in a 24-well plate containing 1 ml of fresh medium and incubated with or without chemical treatment for the indicated time. For cytotoxicity study, the cells were treated with beauvericin during the exponential phase of cell growth.

Measurement of intracellular calcium concentrations. Changes of intracellular calcium concentration ($[Ca^{2+}]_i$) were monitored with the cell-permeable fluorescent calcium indicator, Fluo-3-acetomethoxy ester (Fluo-3/AM). The acetomethoxy ester form of this compound allows the delivery of calcium-insensitive Fluo-3/AM into cells, where the ester is cleaved to produce the active, calcium-sensitive form of Fluo-3. Fluorescence intensity (value) was measured with a Perkin-Elmer Victor 3 fluorescent microplate reader by recording excitation signals at 485 nm and emission signal at 538 nm at data accumulated at 5 s intervals. Maximum and minimum fluorescence values were obtained by adding 0.1% NP and 10 mM EGTA sequentially.

In brief, CCRF-CEM cells (1×10^6) were collected with centrifugation, and incubated in Tyrode's salt solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 0.2 mM NaH_2PO_4, 12 mM $NaHCO_3$, and 5.5 mM glucose) containing 10 μM Fluo-3-AM plus 2% pluronic F-127 for 1 h at room temperature (20–25 °C), and washed in fresh Tyrode's solution to remove unincorporated dye. In some experiments, calcium free Tyrode's solution with 200 μM EGTA (ethyleneglycotetraacetic acid) was used during washing and imaging. CCRF-CEM cells were placed into a 96-well fluorescent microplate (37 °C), the well containing 100 μl Tyrode's solution and 0.1×10^{-6} Fluo-3 pretreated CCRF-CEM cells. The intracellular calcium concentrations ($[Ca^{2+}]_i$) were estimated by measuring the intensity of fluorescence following the equation below [24]:

$$[Ca^{2+}] = K_d[(F - F_{min})/(F_{max} - F)],$$

where K_d is the apparent dissociation constant of Fluo-3 for Ca^{2+} (390 nM), F is the fluorescence intensity, F_{min} is the background fluorescence, and F_{max} is the maximal fluorescence.

Fluorescent images of living cells in Tyrode's solution were acquired using an Olympus Fluorescent Microscope under a 10× objective lens and analyzed.

Other chemicals. Beauvericin was obtained from Dr. Cheng-Jen Chou (National Research Institute of Chinese Medicine). The reagents for the cell culture were obtained from Hyclone. Fluo-3/AM was from Molecular Probes (Eugene, OR, USA). Thapsigargin and nimodipine were purchased from Tocris Cookson (Bristol, USA). Tyrode's solution and other reagents were from Sigma (St. Louis, MO, USA). Agents were dissolved in dimethyl sulfoxide (DMSO) as stock solution. Final concentration of DMSO in the $[Ca^{2+}]_i$ measurements was less than 0.1% and did not alter the basal $[Ca^{2+}]_i$.

Statistical analysis. Data are presented as means ± SEM for the indicated number of experiments. The statistical analysis of data was performed by one-way ANOVA, followed by the Scheffe test and p values less than 0.05 were considered significant.

Results

By using Fluo-3/AM as an indicator, we determined the intracellular calcium concentration ($[Ca^{2+}]_i$). Human lymphoblastic leukemia CCRF-CEM cells loaded with 10 μM Fluo-3/AM were exposed to beauvericin by direct administration to the bathing solution, and the fluorescence intensity was determined before and after treatment.

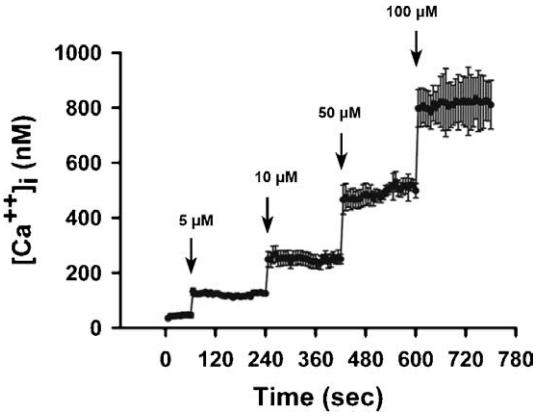

Fig. 2. Effects of beauvericin on $[Ca^{2+}]_i$ in human lymphoblastic leukemia CCRF-CEM cells. The different concentrations of beauvericin (5, 10, 50, and 100 μM) were added into the Ca^{2+}-containing buffer as indicated by the arrows. The $[Ca^{2+}]_i$ in CCRF-CEM cells was monitored constantly. The data are means ± SEM of 5–7 experiments.

An increase in fluorescence intensity was detected 5 s after exposure to beauvericin (Fig. 2). In Ca^{2+}-containing medium, the basal $[Ca^{2+}]_i$ was 60.6 ± 3.82 nM. Beauvericin at concentrations above 5 μM increased $[Ca^{2+}]_i$ in a dose-dependent manner in the presence of extracellular Ca^{2+}. Beauvericin (5 ~ 100 μM) caused an immediate $[Ca^{2+}]_i$ rise and elevated $[Ca^{2+}]_i$ sustained for 180 s after the addition of different concentrations of beauvericin (Fig. 2).

To investigate whether the beauvericin-induced $[Ca^{2+}]_i$ elevation is attributed to the influx of extracellular Ca^{2+} or release of Ca^{2+} from its intracellular stores, we first investigated the effect of beauvericin in the presence of extracellular Ca^{2+}. CCRF-CEM cells were preloaded with 10 μM Fluo-3/AM fluorescent dye prior to beauvericin treatment. A sharply elevated $[Ca^{2+}]_i$ in CCRF-CEM cells in Tyrode's buffer was observed after the addition of beauvericin but not to DMSO used as a vehicle as shown in Fig. 3A. Beauvericin (50 μM) can induce the net $[Ca^{2+}]_i$ rise up to 187.69 ± 26.42 nM ($n = 7$), which is several folds greater than control (DMSO) of 40.02 ± 2.67 nM (Table 1). Beauvericin-induced $[Ca^{2+}]_i$ rise in the cells was prevented by intracellular calcium chelator-BAPTA/AM. Pretreatment of the cells with 10 μM BAPTA/AM reduced beauvericin-induced $[Ca^{2+}]_i$ rise to 58.39 ± 8.45 nM (Fig. 3B, Table 1). On the other hand, pretreatment of the cells with 1 mM $CaCl_2$ in bathing medium increased $[Ca^{2+}]_i$ sharply, up to 251.02 ± 23.14 nM, after beauvericin

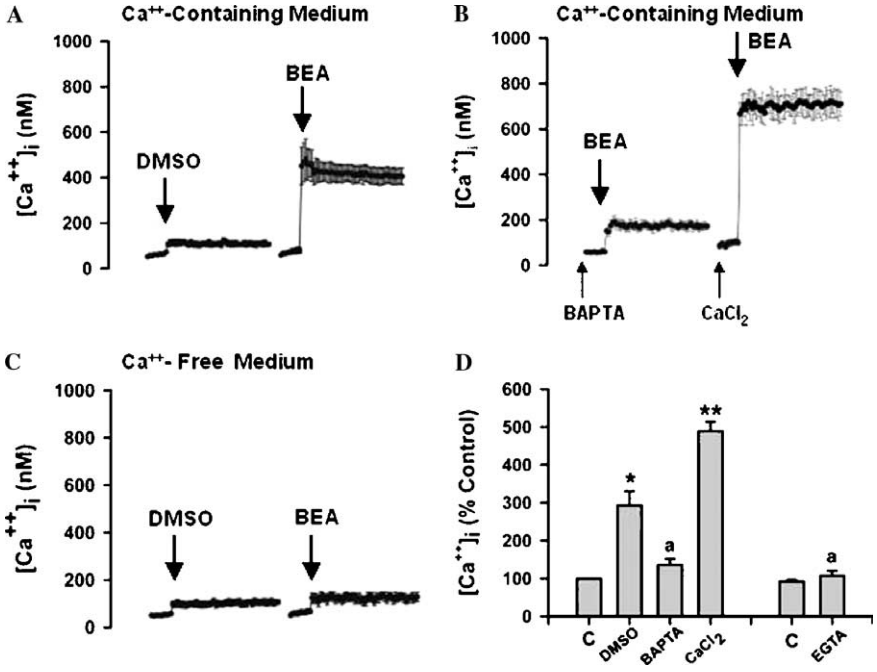

Fig. 3. Study of the sources of $[Ca^{2+}]_i$ rise by beauvericin in human lymphoblastic leukemia CCRF-CEM cells. (A) CCRF-CEM cells were preloaded with Fluo-3/AM fluorescent dye before exposure to beauvericin. Kinetic study of Ca^{2+} mobilization using a Victor 3 (Perkin-Elmer) fluorescent microplate reader. (B) 10 μM BAPTA/AM (an intracellular Ca^{2+} chelator) or 1 mM $CaCl_2$ was pretreated to test the effect on the rise of $[Ca^{2+}]_i$. The experiments were performed in Ca^{2+}-containing buffer. (C) Kinetic study on Ca^{2+} mobilization in human CCRF-CEM leukemia cells suspended in Ca^{2+}-free Tyrode's buffer. The cells were pretreated with 2 mM EGTA. The cells treated with DMSO were used as control and DMSO concentration was less than 0.1%. (D) Comparison of beauvericin-induced $[Ca^{2+}]_i$ with different treatments. The data are expressed as means ± SEM of 5–7 determinations, * $p < 0.01$ and $^{**}p < 0.001$ compared with control group and ^{a} $p < 0.01$ comparison between the pretreatment with BAPTA/AM or DMSO in the same BEA treatment group.

Table 1
Effects of beauvericin on intracellular calcium concentration ($[Ca^{2+}]_i$) in human lymphoblastic leukemia CCRF-CEM cells

Pretreatment	Basal $[Ca^{2+}]_i$ (nM)	Treatment	Net $[Ca^{2+}]_i$ (nM)
Ca^{2+}-containing medium			
None	59.26 ± 1.41	DMSO	40.02 ± 6.27
None	61.72 ± 5.23	Beauvericin	$187.69 \pm 26.42^{*}$
BAPTA	58.29 ± 2.05	Beauvericin	58.39 ± 8.45
$CaCl_2$	95.19 ± 7.29	Beauvericin	$251.02 \pm 23.14^{*}$
Ca^{2+}-free medium			
None	51.56 ± 6.93	DMSO	56.10 ± 4.23
EGTA	50.95 ± 0.72	Beauvericin	$62.18 \pm 3.17^{**}$

The net $[Ca^{2+}]_i$ (nM) is the results of the intracellular calcium concentration ($[Ca^{2+}]_i$) after beauvericin or DMSO treatment minus basal $[Ca^{2+}]_i$. The DMSO-treated cells were used as the control and DMSO concentration was less than 0.1%. The data are expressed as means ± SEM of 5–7 determinations.

* $p < 0.01$ compared with control group.

** $p < 0.01$ comparison between the absence and presence of EGTA in the same beauvericin treatment group.

treatment (Fig. 3B; Table 1). These data suggest that beauvericin caused a sharp extracellular Ca^{2+} influx to raise $[Ca^{2+}]_i$. To further examine that extracellular Ca^{2+} influx possibly attributed to the beauvericin-induced $[Ca^{2+}]_i$ rise, the same experiment was conducted in the absence of extracellular Ca^{2+} ($Ca^{2+}$was substituted with 200 μM EGTA). In Ca^{2+}-free medium, no change in the basal $[Ca^{2+}]_i$ was observed (50.95 ± 0.72 nM). However, the beauvericin-induced $[Ca^{2+}]_i$ rise was diminished, even beauvericin was high, up to 50 μM (Fig. 3C). These data show that removal of extracellular Ca^{2+} inhibited beauvericin-induced $[Ca^{2+}]_i$ rise (62.18 ± 3.17 nM, Fig. 3D, Table 1). As further evidence that the increase in fluorescence intensity after beauvericin exposure was due to changing $[Ca^{2+}]_i$, we also determined the intracellular calcium accumulated by fluorescence microscope. CCRF-CEM cells pretreated with the vehicle control containing 0.1% dimethyl sulfoxide (DMSO) exhibited no fluorescence intensity (Fig. 4A) and beauvericin-treated cells exhibited strong fluorescence intensity (Fig. 4B). BAPTA/AM pretreated had no effect on basal fluorescence and attenuated beauvericin-induced fluorescence levels (Figs. 4C and D). In addition, in Ca^{2+}-free medium, adding 200 μM EGTA in the bathing buffer abolished the beauvericin-induced fluorescence intensity (Figs. 4E and F). Our data suggest that the $[Ca^{2+}]_i$ rise in CCRF-CEM cells is due to the Ca^{2+} influx from the medium or extracellular sources. From these results, we hypothesize that treatment with beauvericin might cause the opening of Ca^{2+} channels, allowing rapid influx of extracellular Ca^{2+}.

To test the hypothesis that treatment with beauvericin might cause the opening of Ca^{2+} channels, L-type Ca^{2+} channel blocker-nimodipine (Nim) was used to block the classic voltage-gated Ca^{2+} channels of CCRF-CEM cells. After pretreatment with DMSO, beauvericin-induced $[Ca^{2+}]_i$ sharp rise for 5 min but DMSO did not cause $[Ca^{2+}]_i$ increase (Figs. 5A and B). As shown in Fig. 5D, beauvericin-induced $[Ca^{2+}]_i$ rise in CCRF-CEM cells in

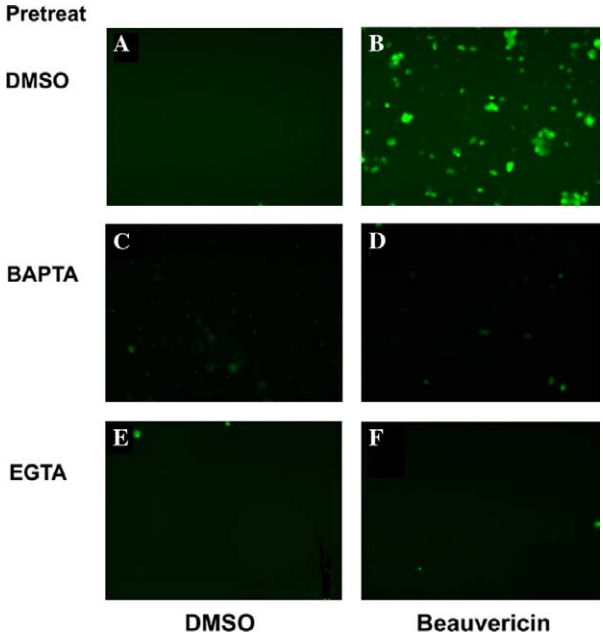

Fig. 4. Fluorescent intensities in CCRF-CEM cells treated with beauvericin under different conditions. Fluorescent intensity in the cells before (left panels) or two minutes after (right panels) treatment with beauvericin, the cells were pretreated with DMSO (upper), BAPTA (middle), or EGTA (lower; in Ca^{2+}-free buffer).

Ca^{2+}-containing medium was not altered by nimodipine (10 μM). Furthermore, the role of the endoplasmic reticulum (ER) Ca^{2+} stores in the beauvericin response was also examined because previous studies have shown that intracellular Ca^{2+} stores may play one of a key role in Ca^{2+} release in various different cells by beauvericin [8,17,18]. In Ca^{2+}-containing medium, pretreatment with 10 μM thapsigargin (TG), an endoplasmic reticulum Ca^{2+} pump inhibitor, did not affect beauvericin-induced $[Ca^{2+}]_i$ rise in CCRF-CEM cells (Fig. 5C).

Discussions

This study is designed to investigate the effect of beauvericin on $[Ca^{2+}]_i$ in human lymphoblastic leukemia CCRF-CEM cells and the attribution of Ca^{2+}sources to beauvericin-induced $[Ca^{2+}]_i$ rise.

Intracellular Ca^{2+} is a key molecule involved in many kinds of signal transduction pathways in a variety of cells. It has been well known that $[Ca^{2+}]_i$ affects cellular secretion, division, growth and differentiation, and is involved in muscle contraction and receptor internalization [25–27]. The results from present study have clearly shown that beauvericin induced significant dose-dependent and sustained increase in $[Ca^{2+}]_i$ at concentrations of 5 μM and above. In Ca^{2+}-containing bathing buffer, the beauvericin-induced $[Ca^{2+}]_i$ elevation did not decline during 5 min of measurement. Sustained increase of $[Ca^{2+}]_i$ has been reported to alter many cellular functions [28]. It is plausible to speculate that beauvericin may significantly affect cell physiology by altering Ca^{2+} signaling and stimulating

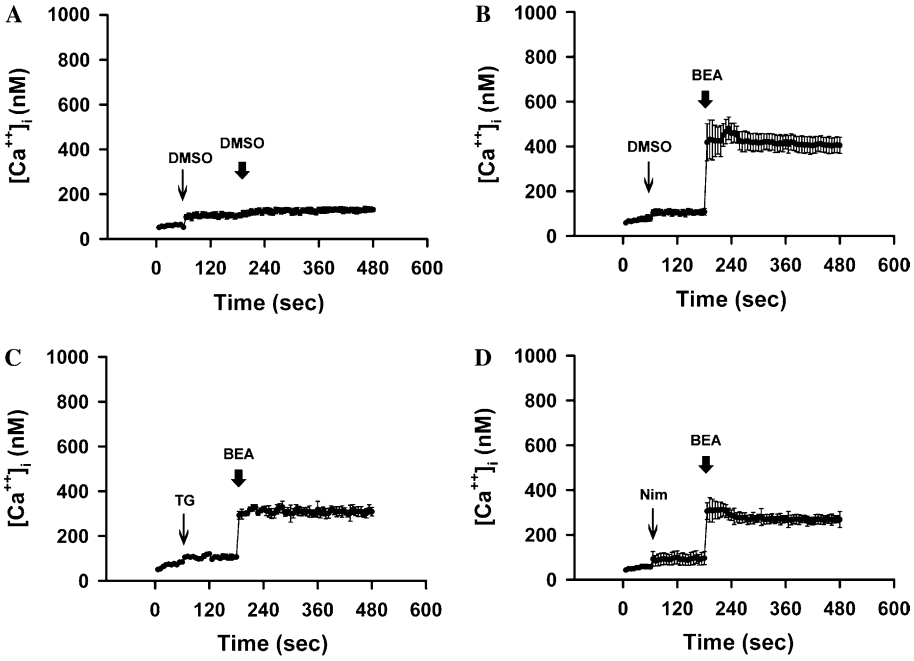

Fig. 5. Beauvericin-induced $[Ca^{2+}]_i$ in CCRF-CEM cells treated with thapsigargin or nimodipine. CCRF-CEM cells were preloaded with Fluo-3/AM fluorescent probe before experiments. CCRF-CEM cells were pretreated (light arrow) with DMSO (A,B), 10 μM thapsigargin (C), or 10 μM nimodipine (D; a voltage-dependent Ca^{2+}-channel blocker) for 2 min. After pretreatment, DMSO (A) or 50 μM beauvericin (B–D) indicated by a black arrow, was added in the bathing solution. The DMSO (<0.1%) treated cells were used as the control. The data are expressed as means ± SEM of 5–7 determinations.

Ca^{2+}-coupled bioactive molecules, which lead to apoptosis in CCRF-CEM cells. Several recent studies have reported the involvement of Ca^{2+} in apoptotic processes [13,14]. The elevated $[Ca^{2+}]_i$ causes proapoptotic protein expression [14]. The rise of $[Ca^{2+}]_i$ has been demonstrated to cause mitochondrial inner transmembrane potential collapse and to induce the release of cytochrome *c* [15]. Interestingly, our previous studies have also showed that beauvericin causes mitochondrial transmembrane potential reduction, release of cytochrome *c*, and then activation of caspase-3 [10]. In addition, we have observed that lowering $[Ca^{2+}]_i$ with an intracellular chelator-(BAPTA/AM) attenuated beauvericin-induced caspase-3 activation and apoptotic death in CCRF-CEM cells [10]. In this study, we have demonstrated that BAPTA directly suppressed beauvericin-induced $[Ca^{2+}]_i$ rise, suggesting that Ca^{2+} may act upstream of caspase-3 activation. These data suggest that beauvericin-induced apoptosis is mediated, at least in part, by Ca^{2+} signaling pathway in the apoptotic process, probably the elevation of $[Ca^{2+}]_i$.

Free intracellular Ca^{2+} can be increased by either extracellular Ca^{2+} influx or the release of stored Ca^{2+} from the intracellular compartment, namely endoplasmic reticulum [29]. Our present results have demonstrated that beauvericin was able to induce an elevated $[Ca^{2+}]_i$ in a dose-dependent manner in human CCRF-CEM leukemia cells incubated in Ca^{2+}-containing medium. Our study results also showed that beauvericin failed to induce an elevated $[Ca^{2+}]_i$ in CCRF-CEM cells as those cells were incubated in $CaCl_2$-free Tyrode's buffer plus EGTA. Furthermore, in Ca^{2+}-containing medium, pretreatment with thapsigargin (TG), an endoplasmic reticulum Ca^{2+} pump inhibitor, did not affect beauvericin-induced $[Ca^{2+}]_i$ rise in CCRF-CEM cells. This result indicated that the endoplasmic reticulum (ER) Ca^{2+} stores were probably not involved in the beauvericin-induced $[Ca^{2+}]_i$ rise although previous studies have shown that intracellular Ca^{2+} stores may play a key role in Ca^{2+} release in various different cells by beauvericin [8,17,18]. Beauvericin-induced $[Ca^{2+}]_i$ rise in CCRF-CEM cells was almost vanished by adding an intracellular chelator-(BAPTA/AM) into Ca^{2+}-containing Tyrode's buffer, but it could be completely restored by adding the extra $CaCl_2$ into the buffer (Fig. 3B). Altogether, these results strongly suggested that intracellular Ca^{2+} which increases $[Ca^{2+}]_i$ in CCRF-CEM cells primarily attributes to the Ca^{2+} influx from extracellular sources.

There are at least two possible mechanisms by which beauvericin can induce the extracellular Ca^{2+}influx. First, beauvericin may indirectly activate endogenous membrane Ca^{2+} channels such as store-operated Ca^{2+} channels [17]. Alternatively, as a recent study suggested in myocytes [9], beauvericin per se may form a cation-selective ion channel. Beauvericin has long been suggested to be a potential ionophore and demonstrated in lipid bilayers and liposomes to form cation-selective channels permeable to K^+, Na^+, and Ca^{2+} [9,20–22]. It is not clearly based on our results from present studies whether beauvericin-induced extracellular Ca^{2+} influx is due to its indirect action of activating endogenous membrane Ca^{2+} channels or its direct action of forming cation-selective ion channels or both in CCRF-

CEM cells. Meanwhile, nimodipine, a blocker of voltage-dependent Ca^{2+} channel, did not have any effect on beauvericin-induced extracellular Ca^{2+} influx, suggesting that the voltage-gated Ca^{2+} channels did not involve in the Ca^{2+} influx.

In summary, this study has demonstrated that beauvericin acts as a potent Ca^{2+} mobilizer to stimulate extracellular Ca^{2+} influx in human acute lymphoblastic leukemia CCRF-CEM cells. Consequently, beauvericin results in a significant increase in $[Ca^{2+}]_i$ in CCRF-CEM cells and it caused a rapid and sustained $[Ca^{2+}]_i$ rise in a dose-dependent manner. Excess extracellular Ca^{2+} facilitated beauvericin-induced $[Ca^{2+}]_i$ rise. In addition, beauvericin-induced $[Ca^{2+}]_i$ rise were attenuated by intracellular Ca^{2+} chelator-BAPTA/AM or in Ca^{2+}-free Tyrode's solution. It is worthy to note that neither the voltage-dependent Ca^{2+} channel blocker, nimodipine, nor depletion of intracellular Ca^{2+} with thapsigargin, an endoplasmic reticulum Ca^{2+} pump inhibitor, has any effect on beauvericin-induced $[Ca^{2+}]_i$ rise. The data from present study indicate that beauvericin acts as a potent Ca^{2+} mobilizer by stimulating extracellular Ca^{2+} influx CCRF-CEM cells.

Acknowledgments

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