啓明醫大學術誌 第28券 2號
Keimyung Med J
Vol. 28, No. 2, December, 2009

Curcumin induces intracellular calcium depletion via a mitochondrial uniporter pathway in PC12 cells*

Jae Hoon Bae, M.D.

Department of Physiology, Keimyung University School of Medicine, Daegu, Korea

Abstract : Curcumin, a natural, biologically active compound extracted from rhizomes of Curcuma species, has been shown to possess the potent properties for regulation of cell proliferation. Although intracellular calcium status is very closely linked with various cellular functions, the action mechanism of curcumin on intracellular calcium signalling remains poorly understood. This study was investigated the effect of curcumin on intracellular calcium concentration ($[Ca^{2+}]_i$) using a calcium indicator dye Fura-2 in PC12 cells. Curcumin induced a marked depletion of $[Ca^{2+}]_i$ in PC12 cells bathed with both Ca^{2+}-containing and Ca^{2+}-free bath solution. To clarify the effects of curcumin on the levels of plasma membrane, extracellular Na^+-free solution and vanadate were applicated on the cells but these did not affect the curcumin effect. Both thapsigargin, a specific inhibitor of sarco/endoplasmic reticulum Ca^{2+}-ATPase, and carbonyl cyanide 3-chlorophenylhydrazone, a mitochondrial uncoupler, also did not change the effect of curcumin on $[Ca^{2+}]_i$. However, ruthenium red, an inhibitor of mitochondrial uniporter, blocked the curcumin-induced $[Ca^{2+}]_i$ depletion in dose-dependent manner with ED_{50} of 2.7 ± 0.14 μM. Present study demonstrates that curcumin induces the intracellular calcium uptake into mitochondria via a uniporter pathway and may involve in the modulation of cellular function and proliferation.

Key Words : Curcumin, Intracellular calcium depletion, Mitochondrial uniporter, Ruthenium red

---

* This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2009-0091361).

Corresponding Author: Jae Hoon Bae, M.D., Department of Physiology, Keimyung University School of Medicine
216, Dalseongno, Jung-gu, Daegu, 700-712 KOREA
Tel: +82-53-250-7452 E-mail: jhbae@dsmc.or.kr

Introduction

Curcumin is the major constituent of turmeric powder extracted from the rhizomes of the plant *Curcuma longa* found in south and southeast tropical Asia. It has been known to possess a wide range of pharmacological properties including anti-inflammatory, hypocholesterolemic and anti-thrombotic activities [1,2]. Several studies in recent years have also shown that curcumin is a potent inhibitor of tumor initiation *in vivo* [3,4] and possesses anti-proliferative activities against tumor cells *in vitro* [5,6]. Curcumin is also one of the potent chemopreventive agents inhibiting tumor promotion against skin, oral, intestinal and colon carcinogenesis [7,8]. It induces several characteristics of apoptosis such as cell shrinkage, chromatin condensation, and DNA fragmentation in various types of cells [9,10].

Changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) stimulate a number of intracellular events and can also trigger a cell death process [11,12]. Calcium has also been implicated in the program of cell death known as apoptosis [13]. Therefore it can be postulated that curcumin would modulate the $[Ca^{2+}]_i$. The limited studies about the relationship between intracellular calcium and curcumin have been reported. Curcumin possibly inhibited Ca^{2+} influx in platelets, as demonstrated by inhibition of platelet aggregation induced by calcium ionophore [1]. In the sarcoplasmic reticulum fraction study of skeletal muscle, curcumin inhibited the Ca^{2+}-ATPase of sarcoplasmic reticulum but increased the rate of accumulation of Ca^{2+} into sarcoplasmic reticulum [14]. However, curcumin-induced $[Ca^{2+}]_i$ changes have not been directly shown in the intact cells.

In order to understand the mechanisms of action of curcumin on modulation in $[Ca^{2+}]_i$, this study was carried out in a rat pheochromocytoma cell line, PC12 cells. The data presented here demonstrate the pharmacodynamics of curcumin inducing the depletion of $[Ca^{2+}]_i$ and the effect of curcumin could be blocked by ruthenium red, a potent mitochondrial uniporter inhibitor, in dose-dependent manner in PC12 cells.

Materials & Methods

Cell isolation and culture

Rat pheochromocytoma (PC12) cell line was obtained from the American Type Culture Collection (Rockville, MD). The PC12 cells were grown in Dulbeco's modified Eagle medium, supplemented with 2 mM glutamine, 10% heat-inactivated fetal bovine serum, 20 mM HEPES buffer and 1% penicillin/streptomycin. Cells were plated on poly-D-lysin-coated glass coverslip (25 mm in diameter) at a density of 2×10^5 cells for measurement of $[Ca^{2+}]_i$. Cells were incubated in a humidified atmosphere (5% CO_2, 95% air) at 37℃ and medium changed 3 times per week.

Materials and solutions

Dulbeco's modified Eagle medium and other supplements were obtained from GibcoBRL (Grand Island, NY). Curcumin and poly-D-lysin (Sigma, St. Louis, MO) dissolved in ethanol and deionized distilled water as a stock solution (100 mM and 5

mg/ml, respectively) and then used at the final concentration in the testing solution. Fura-2 acetoxymethyl ester (Fura-2/AM) was obtained from Molecular Probes (Eugene, OR) and dissolved in dimethyl sulfoxide. Ruthenium red was dissolved in deionized distilled water, and thapsigargin and carbonyl cyanide 3-chlorophenylhydrazone (CCCP) were dissolved in dimethyl sulfoxide. All other laboratory chemicals were purchased from Sigma Chemical Co.

Physiological saline solution (PSS) for measurement of $[Ca^{2+}]_i$ contained (in mM): NaCl (126), KCl (5), $CaCl_2$ (1), $MgCl_2$ (1.2) HEPES (10), D-glucose (10). The solution was titrated to pH 7.4 with HCl and osmorality was 290 mOsm. When Ca^{2+}-free solution was used, Ca^{2+} was omitted and 2 mM EGTA was added. To block the plasma membrane Na^+-Ca^{2+} exchange, external Na^+ was replaced by N-methyl-D-glucamine.

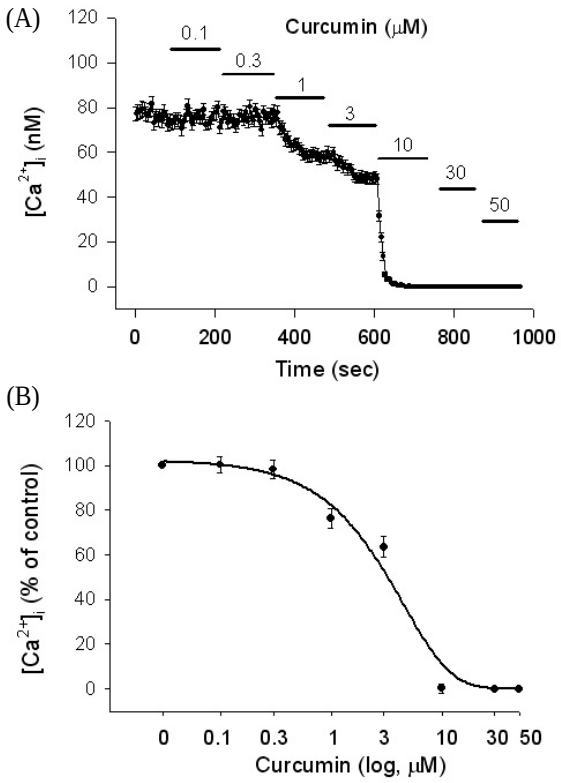
Measurement of intracellular calcium concentration

Microfluorescent imaging of $[Ca^{2+}]_i$ was performed on PC12 cells loaded with the calcium indicator dye Fura-2/AM. Some of the procedures used in calcium imaging in this experiment have been described [15,16]. Fura-2/AM (3 μM) was added to PC12 cells bathed in PSS at room temperature for 30 min followed by a 30-min wash in dye-free PSS to allow esterase conversion to free Fura-2. Coverslips were placed on the stage of an inverted microscope and imaging was performed with an InCa dual-wavelength system (Intracellular Imaging, Cincinnati, OH). $[Ca^{2+}]_i$ was calculated as the relationship between the ratio of emissions at 510 nm from excitation at 340 and 380 nm. Ratio images were processed every 5 s and converted to $[Ca^{2+}]_i$ as compared to a range of such ratios obtained by measurement of Fura-2 in the presence of known concentration of calcium (Calcium Calibration Buffer Kit, Molecular Probe). Each experimental data point represents the mean value of $[Ca^{2+}]_i$ calculated from at least 12 individually measured PC12 cells from three separate cultures. All imaging experiments were done at room temperature (20-22℃). The results in the figure and text are given as mean ± SEM.

Results

Effects of curcumin on $[Ca^{2+}]_i$ in the presence and absence of extracellular calcium in PC12 cells

To verify whether curcumin may change the intracellular calcium status, $[Ca^{2+}]_i$ were measured after application of various range in concentrations of curcumin. It induced a marked depletion in $[Ca^{2+}]_i$ over the concentration range of 1 μM curcumin in PC12 cells perfused with Ca^{2+}-contained standard PSS (Fig. 1A). To examine the dose-dependent relationship of curcumin-induced $[Ca^{2+}]_i$ depletion, curcumin was treated sequentially with increasing concentration in PSS from 0.1 μM to 50 μM. The curcumin effect showed a maximal response at 10 μM of the concentrations (Fig. 1B). In Fig 1B, the ED_{50} of the curcumin-induced $[Ca^{2+}]_i$ depletion calculated from 51 cells was 3.9 ± 0.56 μM. This experiment



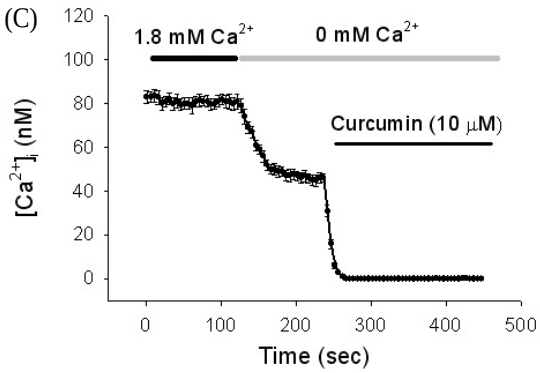


Fig. 1. Effect of curcumin on $[Ca^{2+}]_i$ in PC12 cells bathed with Ca^{2+}-containing or Ca^{2+} free solution. (A) Curcumin induced dose-dependent $[Ca^{2+}]_i$ depletion under 1.8 mM Ca^{2+}-containing standard physiological saline solution (PSS). (B) Curcumin decreased $[Ca^{2+}]_i$ in Ca^{2+}-containing PSS in a dose-dependent manner. ED_{50} was 3.9 ± 0.56 μM (n=51). Relative dose responses were compared with changes (%) to control values before curcumin treatment. (C) Curcumin (10 μM)-induced $[Ca^{2+}]_i$ depletion in PC12 cells loaded with Ca^{2+}-free (containing 2 mM EGTA) solution. The absence of extracellular Ca^{2+} did not affect the $[Ca^{2+}]_i$ depletion induced by curcumin. Data are mean ± SEM of measurements made in 17 PC12 cells (A and C) and in three independent experiments (B), respectively.

was further examined to explore the effect of curcumin in Ca^{2+}-free solution. Curcumin (10 μM) also completely abolished the $[Ca^{2+}]_i$ in PC12 cells loaded with 0 mM Ca^{2+} and 2 mM EGTA-containing solution (Fig. 1C). Ca^{2+}-free experiment did not show any difference in curcumin-induced calcium response depend on the extracellular Ca^{2+} influx in PC12 cells.

Effects of inhibition of plasma membrane Na^+-Ca^{2+} exchanger and Ca^{2+}-ATPase on the curcumin-induced $[Ca^{2+}]_i$ depletion in PC12 cells

To rule out $[Ca^{2+}]_i$ lowering pathways on plasma membrane in PC12 cells, this experiment was tried to block the Na^+-Ca^{2+} exchanger by replacement of external Na^+ with N-methyl-D-glucamine or inhibit Ca^{2+}-ATPase by vanadate. Curcumin could still lower $[Ca^{2+}]_i$ at 10 μM and completely vanished it at 30 μM in the extracellular free-Na^+ solution (Fig. 2A). Sodium orthovanadate (1 mM), an inhibitor of plasma membrane Ca^{2+}-ATPase, failed to eliminate the curcumin-induced depletion of $[Ca^{2+}]_i$ under standard PSS (Fig. 2B). These results suggest that curcumin-induced $[Ca^{2+}]_i$ depletion was not due to an activation of Ca^{2+} efflux via both plasma membrane Ca^{2+}-

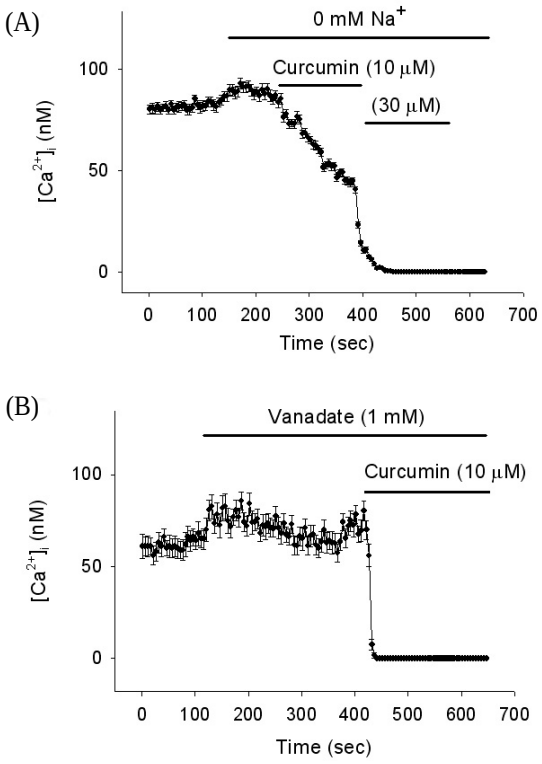


Fig. 2. Effects of inhibitors of plasma membrane Na^+-Ca^{2+} exchanger and Ca^{2+}-ATPase on curcumin-induced $[Ca^{2+}]_i$ depletion in Ca^{2+}-containing PSS. (A) Blockade of plasma membrane Na^+-Ca^{2+} exchanger (replacing of external Na^+ with N-methyl-D-glucamine) had no effect on curcumin-induced $[Ca^{2+}]_i$ depletion. (B) Inhibitor of plasma membrane Ca^{2+}-ATPase, 1 mM vanadate, did not affect curcumin-induced $[Ca^{2+}]_i$ depletion. Data are mean ± SEM of measurements made in 20-30 PC12 cells.

ATPase and Na^+−Ca^{2+} exchanger.

Effects of intracellular calcium uptake inhibitors on curcumin-induced $[Ca^{2+}]_i$ depletion in PC12 cells

This study was also tried to examine the role of endoplasmic reticulum and mitochondria as important intracellular calcium restoring organelles on curcumin-induced $[Ca^{2+}]_i$ depletion in PC12 cells. Upon exposure to 2 μM thapsigargin, a specific inhibitor of sarco/endoplasmic reticulum Ca^{2+}−ATPase (SERCA), in standard PSS, $[Ca^{2+}]_i$ was typically increased and then slowly decreased to a higher level of baseline. At this point the addition of 10 μM curcumin induced a complete depletion in $[Ca^{2+}]_i$ (Fig. 3A). Under 10 μM CCCP, an inhibitor of the rapid mode of mitochondrial Ca^{2+} uptake [17] as well as the mitochondrial uncoupler [18], curcumin also induced the complete $[Ca^{2+}]_i$ depletion in the same extent (Fig. 3B). This means both SERCA and rapid mode of mitochondrial Ca^{2+} uptake seem not to participate in curcumin−induced decreasing $[Ca^{2+}]_i$. However, the addition of ruthenium red (10 μM), a mitochondrial uniporter inhibitor, blocked the $[Ca^{2+}]_i$ depletion induced by 10 μM curcumin, and it could only attenuate the response of 50 μM curcumin in PC12 cells (Fig. 3C). This finding suggests that the mitochondrial uniporter, another mitochondrial Ca^{2+} uptake pathway, should be involved in a major contributory mechanism of curcumin−induced $[Ca^{2+}]_i$ depletion.

Ruthenium red attenuates the curcumin-induced $[Ca^{2+}]_i$ depletion in PC12 cells

This experiment was further extended to examine the effect of ruthenium red on the curcumin−induced $[Ca^{2+}]_i$ responses after application of ionomycin, an Ca^{2+} ionophore, for magnifying action of ruthenium red. Application of 10 μM ionomycin induced a large and sustained increase in $[Ca^{2+}]_i$ and reached at a plateau level in standard PSS. At the plateau state, co−application of 10 μM curcumin with ionomycin induced a complete

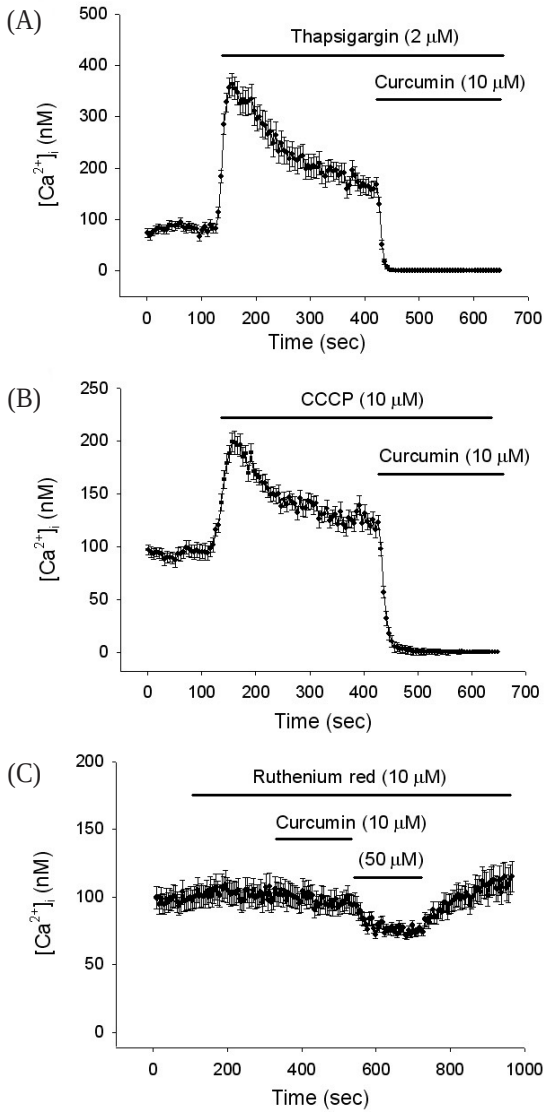


Fig. 3. Effects of intracellular calcium uptake inhibitors on curcumin-induced $[Ca^{2+}]_i$ depletion in Ca^{2+}-containing PSS. An addition of 10 μM curcumin induced a complete depletion in $[Ca^{2+}]_i$ under 2 μM thapsigargin, a SERCA inhibitor (A) or 10 μM CCCP, an inhibitor of rapid mode of mitochondrial Ca^{2+} uptake as well as mitochondrial uncoupler (B). (C) An application of ruthenium red (10 μM), a mitochondrial uniporter inhibitor, blocked the $[Ca^{2+}]_i$ depletion induced by 10 μM curcumin and attenuated the response after addition of 50 μM curcumin. Data are mean ± SEM of measurements made in 20-30 PC12 cells.

$[Ca^{2+}]_i$ depletion within 5 min (Fig. 4A). To verify the dose-response relationship of ruthenium red on the attenuation of curcumin-induced $[Ca^{2+}]_i$ depletion, the various concentrations of ruthenium red were added into bath solution with ionomycin before application curcumin, as in Fig 4B. Ruthenium red apparently attenuated the $[Ca^{2+}]_i$ depletion induced by 10 μM curcumin in dose-dependent manner (Fig. 4B). Curcumin induced a complete $[Ca^{2+}]_i$ depletion under ruthenium red-free solution, but this $[Ca^{2+}]_i$ depletion was gradually minimized by the increment of ruthenium red concentrations. The attenuating effect of ruthenium red on curcumin-induced $[Ca^{2+}]_i$ depletion showed a maximal response at 30 μM ruthenium red (Fig. 4C). The ED_{50} of ruthenium red effect calculated from 37 cells was 2.7 ± 0.14 μM. These findings apparently suggest that curcumin depletes $[Ca^{2+}]_i$ through the mitochondrial Ca^{2+} uptake pathway, and the $[Ca^{2+}]_i$ depletion is blocked by ruthenium red, a potent inhibitor of mitochondrial uniporter, in PC12 cells.

Ruthenium red and curcumin *per se* did not affect the basal Ca^{2+} concentration and ruthenium red returns the curcumin-induced $[Ca^{2+}]_i$ depletion to baseline level

The solution of ruthenium red and curcumin have specific red and yellow colors, respectively. It should be tested to rule out whether an addition of these colored solution may affect the light ratios emitted from the free Fura-2 pentapotassium containing bath solution. Like as our expectation, curcumin and/or ruthenium red did not change the

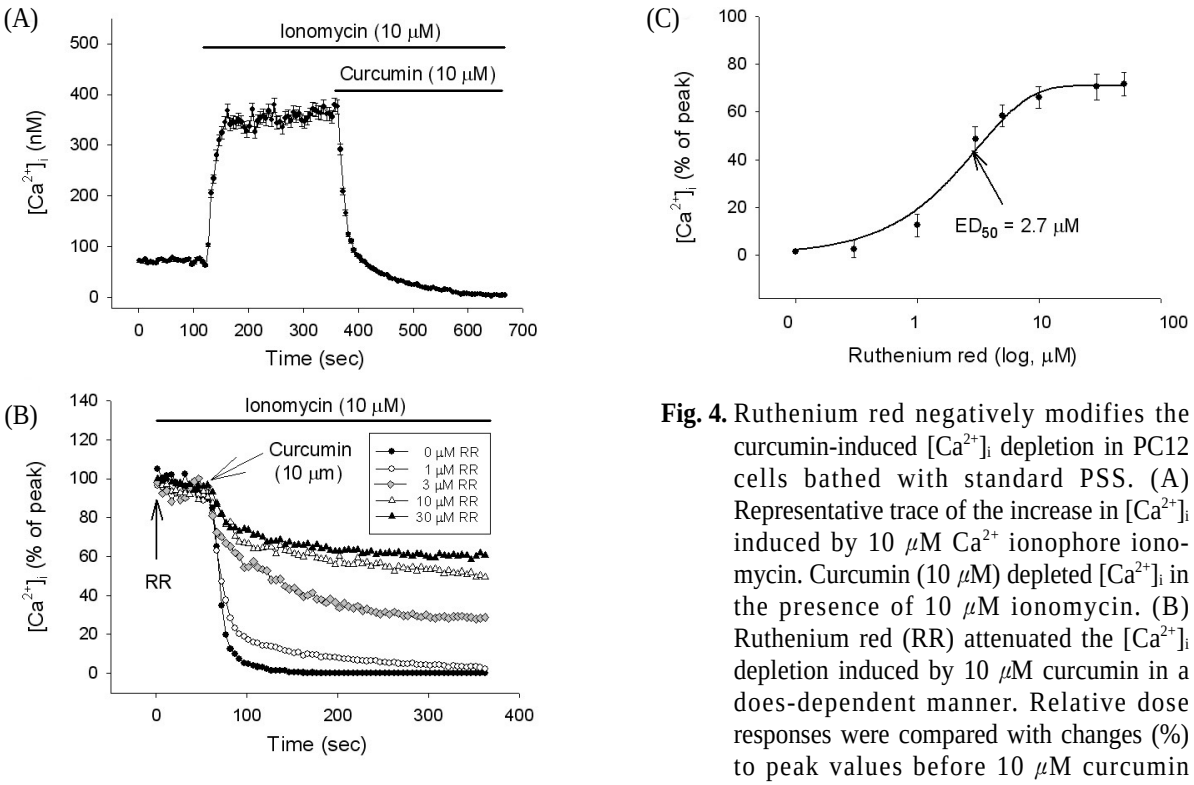


Fig. 4. Ruthenium red negatively modifies the curcumin-induced $[Ca^{2+}]_i$ depletion in PC12 cells bathed with standard PSS. (A) Representative trace of the increase in $[Ca^{2+}]_i$ induced by 10 μM Ca^{2+} ionophore ionomycin. Curcumin (10 μM) depleted $[Ca^{2+}]_i$ in the presence of 10 μM ionomycin. (B) Ruthenium red (RR) attenuated the $[Ca^{2+}]_i$ depletion induced by 10 μM curcumin in a does-dependent manner. Relative dose responses were compared with changes (%) to peak values before 10 μM curcumin treatment in the presence of 10 μ M ionomycin and ruthenium red. (C) Dose-response relationship of ruthenium red on attenuation of curcumin-induced $[Ca^{2+}]_i$ depletion. ED_{50} of ruthenium red effect was 2.7 $\pm$ 0.14 μM (n=37). Data are mean $\pm$ SEM of measurements made in 20-30 PC12 cells.

baseline Ca^{2+} concentration elicited by standard 100 nM Ca^{2+} bath solution (Fig. 5A). These findings revealed that two compounds per se did not induce any interference in the change of Ca^{2+} response and the curcumin and/or ruthenium red-induced Ca^{2+} results are not artifact. Finally this experiment also tried to reconfirm the effect of ruthenium red on previously depleted calcium level induced by curcumin. Ruthenium red also slowly returned the curcumin-induced $[Ca^{2+}]_i$ depletion to the baseline Ca^{2+} level (Fig. 5B).

Discussion

In this study, curcumin decreases $[Ca^{2+}]_i$ in a dose-dependent manner and induces the complete depletion of $[Ca^{2+}]_i$ in PC12 cells over the concentration of 10 μM. Extrusion, uptake and/or buffering of Ca^{2+} should be able to account for this depletion of $[Ca^{2+}]_i$ [18]. The experiment was tried to explore the several different mechanisms: (a) inhibition of Ca^{2+} influx; (b) activation of Ca^{2+} extrusion via Na^+-Ca^{2+} exchanger on plasma membrane; (c) activation of Ca^{2+} extrusion via plasma membrane Ca^{2+}-ATPase; (d) activation of Ca^{2+} uptake mechanism into endoplasmic reticulum via SERCA; (e) activation of Ca^{2+} uptake into mitochondria [18].

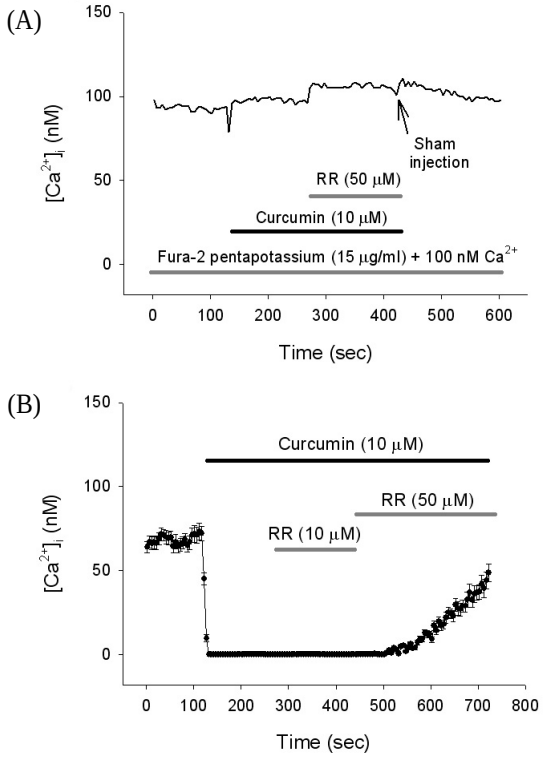


Fig. 5. Curcumin and ruthenium red (RR) does not change the basal Ca^{2+} level (A). Fura-2 pentapotassium (15 μg/ml) in 100 nM standard Ca^{2+} solution showed a constant $[Ca^{2+}]$ level which was not affected by an addition of curcumin and/or RR. RR recovers the depleted $[Ca^{2+}]_i$ level induced by curcumin (B). The depleted $[Ca^{2+}]_i$ level previously induced by curcumin was slowly returned to the baseline level according to co-treatment with RR (50 μM). Data are mean ± SEM of measurements made in 15-25 PC12 cells.

Firstly, it was tested to clarify the possible action mechanisms of curcumin on the level of plasma membrane. The Ca^{2+}-free solution, in the inhibition of Ca^{2+} influx from extracellular solution, could not mask the curcumin-induced depletion effect, therefore the blocking effect on Ca^{2+} influx was independent from the action mechanism of curcumin. It was also needed to check the curcumin effect on Ca^{2+} extrusion to extracellular space. Under inhibition of Na^+-Ca^{2+} exchanger [19], the Na^+-free solution had no effect. An inhibitor of plasma membrane Ca^{2+}-ATPase, vanadate [18], also did not show any changes. Therefore, it should be valid that curcumin-induced $[Ca^{2+}]_i$ depletion was not due to a mechanism on the plasma membrane.

Other possible pathways for curcumin-induced $[Ca^{2+}]_i$ depletion are the sequestration of Ca^{2+} into the intracellular Ca^{2+} storing organelles, endoplasmic reticulum and/or mitochondria [18]. Therefore it was tried to check the possible involvement of SERCA on the curcumin-induced $[Ca^{2+}]_i$ depletion, but thapsigargin did not block the curcumin effect. It seems that the curcumin-induced $[Ca^{2+}]_i$ depletion differs with the Ca^{2+} uptake via activation of SERCA in PC12 cells.

Curcumin has been attracted the attention of many scientists since it is shown to display anti-carcinogenesis properties in a wide variety of cell lines [9,20] and in animals [7,21]. It has been reported that curcumin has ability to induce apoptosis of tumor cells [2,22] and apoptosis of HL-60 [23] and U937 [6] leukemia cells. Multiple evidences suggest that mitochondria might be involved in the induction of apoptosis [24].

Mitochondria are also a controller of intracellular Ca^{2+} dynamics and transport through a complex system consisting of two modes of influx and two of efflux [17,25]. Among them, mitochondrial Ca^{2+} influx pathways could be participated in the curcumin-induced $[Ca^{2+}]_i$ depletion. The most thoroughly studied Ca^{2+} influx mechanism is the uniporter, which facilitates the diffusion of Ca^{2+} via lowering its electrochemical gradient and does not couple the transport to that of

any other ions of molecule [25]. This pathway has often been referred to the ruthenium red-sensitive uptake pathway [26]. In addition to this uniporter, another mode of Ca^{2+} influx into mitochondria has been described recently as rapid uptake mode. The rapid uptake pathway permits Ca^{2+} across the inner membrane for brief periods within about 20 ms and is inhibited very soon after it is begun [17,25—27]. This rapid mode is inhibited by mitochondrial uncoupler, CCCP, which dissipates the mitochondrial membrane potential to near zero and collapses Ca^{2+} uptake into mitochondria driven by the membrane potential [17,28]. In this experiment, CCCP had no effect on attenuating the curcumin-induced $[Ca^{2+}]_i$ depletion in PC12 cells. Therefore it was further needed to explore the another activation of mitochondrial uniporter pathway, whether or not it was participated in the $[Ca^{2+}]_i$ depletion of curcumin.

Ruthenium red, a hexavalent polysaccharide stain, particularly for glycoproteins, is a non-competitive inhibitor of mitochondrial Ca^{2+} uniporter and abolishes Ca^{2+} influx [17,29]. In present study, ruthenium red (10 μM) blocked the $[Ca^{2+}]_i$ depletion induced by 10 μM curcumin. In addition to a uniporter inhibitor, ruthenium red is also an inhibitor of ryanodine-sensitive Ca^{2+} release channel from endoplasmic reticulum, an intracellular Ca^{2+} store [30]. It was also tried to verify the inhibitory effect of ruthenium red on Ca^{2+} release from intracellular calcium storing organelles by using another inhibitor of ryanodine receptor, dantrolene sodium [31]. In the presence of 10 μM dantrolene sodium, curcumin induced the complete $[Ca^{2+}]_i$ depletion (data not shown), showing the unique effect of ruthenium red on curcumin-induced Ca^{2+} response was only possible by the inhibitory action on mitochondrial uniporter system. It represents apparently that curcumin induces the $[Ca^{2+}]_i$ depletion through the cytosolic Ca^{2+} uptake via mitochondrial uniporter. Normally, most of intracellular Ca^{2+} resides within the lumen of the endoplasmic reticulum, with very little in the mitochondria. If the Ca^{2+} stored within endoplasmic reticulum was depleted, the mitochondria would become overloaded and there would initiate a programme of events that leads to cell death called apoptosis [13]. Recently, our colleague observed that ruthenium red specially inhibited curcumin-induced apoptosis and markedly prevented the activation of caspase 3, cytochrome *c* release and cell death in the curcumin treated U937 cells [6]. In addition to these cytoprotective effects, ruthenium red attenuated the curcumin-induced $[Ca^{2+}]_i$ depletion in renal cell tumor Caki cells [6] same as this experimental results in PC12 cells.

In conclusion, the findings in this study demonstrated that curcumin induces $[Ca^{2+}]_i$ depletion under both Ca^{2+}-containing and Ca^{2+}-free solution in PC12 cells. The $[Ca^{2+}]_i$ depletion is only blocked by ruthenium red, an inhibitor of mitochondrial uniporter, in dose-dependent manner. These results suggest that curcumin modulates $[Ca^{2+}]_i$ and acts as an stimulator of intracellular Ca^{2+} uptake into mitochondria via uniporter pathway.

Summary

This stuudy was investigated to clarify the effect of curcumin on intracellular calcium

concentration ($[Ca^{2+}]_i$) in PC12 cells. Curcumin induced a marked depletion of $[Ca^{2+}]_i$ in PC12 cells bathed with both Ca^{2+}-containing and Ca^{2+}-free solution. Extracellular Na^+-free solution and vanadate did not inhibit the curcumin effect on the level of plasma membrane. Thapsigargin and carbonyl cyanide 3-chlorophenylhydrazone also had no effect and both SERCA and mitochondrial rapid Ca^{2+} uptake mode did not participate in curcumin-induced calcium depletion. Ruthenium red, an inhibitor of mitochondrial uniporter, only attenuated the curcumin-induced $[Ca^{2+}]_i$ depletion in dose-dependent manner with ED_{50} of 2.7 ± 0.14 μM. Present study demonstrates that curcumin acts as a stimulator of intracellular Ca^{2+} uptake into mitochondria via uniporter pathway and may involve in the modulation of cellular function.

References

1. Shah BH, Nawaz Z, Pertani SA, Roomi A, Mahmood H, Saeed SA, *et al*. Inhibitory effect of curcumin, a food spice from turmeric, on platelet-activating factor- and arachidonic acid-mediated platelet aggregation through inhibition of thromboxane formation and Ca^{2+} signalling. *Biochem Pharmacol* 1999;**58**:1167-72.
2. Jagtap S, Meganathan K, Wagh V, Winkler J, Hescheler J, Sachinidis A. Chemoprotective mechanism of the natural compounds, epigallocatechin-3-O-gallate, quercetin and curcumin against cancer and cardiovascular diseases. *Curr Med Chem* 2009;**16**:1451-62.
3. López-Lázaro M. Anticancer and carcinogenic properties of curcumin: considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent. *Mol Nutr Food Res* 2008;**52**:S103-27.
4. Huang MT, Newmark HL, Frenkel K. Inhibitory effects of curcumin on tumorigenesis in mice. *J Cell Biochem Suppl* 1997;**27**:26-34.
5. Mehta K, Pantazis P, McQueen T, Aggarwal BB. Antiproliferative effect of curcumin (diferuloylmethane) against human breast tumor cell lines. *Anticancer Drugs* 1997;**8**:470-81.
6. Bae JH, Park JW, Kwon TK. Ruthenium red, inhibitor of mitochondrial Ca^{2+} uniporter, inhibits curcumin-induced apoptosis via the prevention of intracellular Ca^{2+} depletion and cytochrome *c* release. *Biochem Biophys Res Commun* 2003;**303**:1073-9.
7. Kuttan G, Kumar KB, Guruvayoorappan C, Kuttan R. Antitumor, anti-invasion, and antimetastatic effects of curcumin. *Adv Exp Med Biol* 2007;**595**:173-84.
8. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Sigman CC. Chemopreventive drug development: perspectives and progress. *Cancer Epidemiology Biomarkers Prev* 1994;**3**:85-98.
9. Jiang MC, Yang-Yen HF, Lin JK, Yen JJ. Differential regulation of p53, c-Myc, Bcl-2 and Bax protein expression during apoptosis induced by widely divergent stimuli in human hepatoblastoma cells. *Oncogene* 1996;**13**:609-16.
10. Lin JK. Molecular targets of curcumin. *Adv Exp Med Biol* 2007;**595**:227-43.
11. Johnson EM Jr, Koike T, Franklin J. A "calcium set-point hypothesis" of neuronal dependence on neurotrophic factor. *Exp Neurol* 1992;**115**:163-6.
12. Berridge MJ, Lipp P, Bootmanm MD. The versatility and universality of calcium signaling. *Nat Rev Mol Cell Biol* 2000;**1**:11-21.
13. Berridge MJ, Bootman MD, Lipp P. Calcium-a life and death signal. *Nature* 1998;**395**:645-8.
14. Logan-Smith MJ, Lockyer PJ, East JM, Lee AG. Curcumin, a molecule that inhibits the Ca^{2+}-ATPase of sarcoplasmic reticulum but increases the rate of

accumulation of Ca^{2+}. *J Biol Chem* 2001;**276**:46905-11.

15. McLarnon JG, Franciosi S, Wang X, Bae JH, Choi HB, Kim SU. Acute actions of tumor necrosis factor-α on intracellular Ca^{2+} and K^{+} currents in human microglia. *Neuroscience* 2001:**104**;1175-84.
16. Bae JH, Mun KC, Park WK, Lee SR, Baek WK, Yim MB, *et al*. EGCG attenuates AMPA-induced intracellular calcium increase in hippocampal neurons. *Biochem Biophys Res Commun* 2002;**290**:1506-12.
17. Gunter TE, Buntinas L, Sparagna G, Eliseev R, Gunter K. Mitochondrial calcium transport: mechanisms and functions. *Cell Calcium* 2000;**28**:285-96.
18. Salathe M, Ivonnet PI, Lieb T, Bookman RJ. Agonist-stimulated calcium decreases in ovine ciliated airway epithelial cells: role of mitochondria. *J Physiol* 2001;**531**:13-26.
19. Sidky AO, Baimbridge KG. Calcium homeostatic mechanisms operating in cultured postnatal rat hippocampal neurones following flash photolysis of nitrophenyl-EGTA. *J Physiol* 1997;**504**:579-90.
20. Surh YJ, Chun KS. Cancer chemopreventive effects of curcumin. *Adv Exp Med Biol* 2007;**595**:149-72.
21. Mahmoud NN, Carothers AM, Grunberger D, Bilinski RT, Churchill MR, Martucci C, *et al*. Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. *Carcinogenesis* 2000;**21**:921-7.
22. Shishodia S, Chaturvedi MM, Aggarwal BB. Role of curcumin in cancer therapy. *Curr Probl Cancer* 2007;**31**:243-305.
23. Kuo ML, Huang TS, Lin JK. Curcumin, an antioxidant and anti-tumor promoter, induces apoptosis in human leukemia cells. *Biochim Biophys Acta* 1996;**1317**:95-100.
24. Pinton P, Giorgi C, Siviero R, Zecchini E, Rizzuto R. Calcium and apoptosis: ER-mitochondria Ca^{2+} transfer in the control of apoptosis. *Oncogene* 2008;**27**:6407-18.
25. Gunter TE, Yule DI, Gunter KK, Eliseev RA, Salter JD. Calcium and mitochondria. *FEBS Lett* 2004;**567**:96-102.
26. Duchen MR, Verkhratsky A, Muallem S. Mitochondria and calcium in health and disease. *Cell Calcium* 2008;**44**:1-5.
27. Celsi F, Pizzo P, Brini M, Leo S, Fotino C, Pinton P, Rizzuto R. Mitochondria, calcium and cell death: a deadly triad in neurodegeneration. *Biochim Biophys Acta* 2009;**1787**:335-44.
28. Pariente J.A, Camello C, Camello PJ, Salido GM. Release of calcium from mitochondrial and nonmitochondrial intracellular stores in mouse pancreatic acinar cells by hydrogen peroxide. *J Membr Biol* 2001;**179**:27-35.
29. Hajnóczky G, Csordás G, Das S, Garcia-Perez C, Saotome M, Sinha Roy S, *et al*. Mitochondrial calcium signalling and cell death: approaches for assessing the role of mitochondrial Ca^{2+} uptake in apoptosis. *Cell Calcium* 2006;**40**:553-60.
30. Ehrlich BE, Kaftan E, Bezprozvannaya S, Bezprozvanny I. The pharmacology of intracellular Ca^{2+}-release channels. *Trends Pharmacol Sci* 1994;**15**:145-9.
31. Belde PJ, Vossen JH, Borst-Pauwels GW, Theuvenet AP. Inositol 1,4,5-trisphosphate releases Ca^{2+} from vacuolar membrane vesicles of Saccharomyces cerevisiae. *FEBS Lett* 1993;**323**:113-8.