Chinese Journal of Physiology 59(5): 268-275, 2016
DOI: 10.4077/CJP.2016.BAF421

Effect of NPC15199 on $[Ca^{2+}]_i$ and Viability in SCM1 Human Gastric Cancer Cells

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Abstract

NPC15199 is a synthesized compound that inhibits inflammation in some models. However, whether NPC15199 affects Ca^{2+} homeostasis in human gastric cancer is unclear. This study examined the effect of NPC15199 on cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]_i$) and viability in SCM1 human gastric cancer cells. The Ca^{2+}-sensitive fluorescent dye fura-2 was used to measure $[Ca^{2+}]_i$. NPC15199 evoked $[Ca^{2+}]_i$ rises concentration-dependently. The response was reduced by removing extracellular Ca^{2+}. NPC15199-evoked Ca^{2+} entry was not inhibited by store-operated channel inhibitors (nifedipine, econazole and SKF96365) and protein kinase C (PKC) activator (phorbol 12-myristate 13 acetate, PMA), or PKC inhibitor (GF109203X). In Ca^{2+}-free medium, treatment with the endoplasmic reticulum Ca^{2+} pump inhibitor thapsigargin or 2,5-di-tert-butylhydroquinone (BHQ) nearly abolished NPC15199-evoked $[Ca^{2+}]_i$ rises. Conversely, treatment with NPC15199 also nearly abolished thapsigargin or BHQ-evoked $[Ca^{2+}]_i$ rises. Inhibition of phospholipase C (PLC) with U73122 did not affect NPC15199-evoked $[Ca^{2+}]_i$ rises. NPC15199 at concentrations of 100-900 μM induced concentration-dependent, Ca^{2+}-independent decrease in viability. Together, in SCM1 cells, NPC15199 induced $[Ca^{2+}]_i$ rises that involved Ca^{2+} entry through PKC-insensitive non-store-operated Ca^{2+} channels and PLC-independent Ca^{2+} release from the endoplasmic reticulum. NPC15199 also induced Ca^{2+}-independent cell death.

Key Words: Ca^{2+}, endoplasmic reticulum, fura-2, gastric cancer cells, NPC15199

Introduction

N-(fluorenyl-9-methoxycarbonyl) amino acids (such as NPC15199: leumedin N-(fluorenyl-9-methoxycarbonyl)-leucine), are a class of anti-inflammatory agents (5). NPC15199 has been used

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Received: January 13, 2016; Revised: March 3, 2016; Accepted: March 22, 2016.
 ISSN : 0304-4920. http://www.cps.org.tw

in vivo to attenuate ileitis (16, 17). In addition to anti-inflammatory effects, NPC15199 was shown to reduce rat mammary gland cacrinogenesis (1) and delay breast cancer in a mouse model (18). Furthermore, *in vitro* studies, NPC15199 played an important role in Ca^{2+} signaling in different cell models such as bladder female transitional carcinoma (BFTC) cells (13) and Madin-Darby canine kidney (MDCK) cells (12). However, its effect on other cell types and the differences in mechanisms between cell types are unknown.

A change in cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]_i$) is a crucial regulator of diverse cellular processes (8, 9). To achieve a precise regulation of $[Ca^{2+}]_i$ and related signaling pathways, cells have many mechanisms to control $[Ca^{2+}]_i$. Many Ca^{2+} channels are characterized by the presence of seven transmembrane domains (8, 9). Activation of these receptors usually activate phospholipase C (PLC) resulting in Ca^{2+} release from stores, which in turn induces Ca^{2+} entry across the plasma membrane (8, 9). The effect of NPC15199 on $[Ca^{2+}]_i$ and viability in human gastric cancer cells is unclear. Thus the aim of the present study was to explore the effect of NPC15199 on $[Ca^{2+}]_i$ and viability in SCM1 human gastric cancer cells. This cell line is a useful model for gastric cancer research. It has been shown that in this cell, several ligands can cause $[Ca^{2+}]_i$ rises, such as thimerosal (15) and caffeic acid (6), *via* causing both Ca^{2+} entry and Ca^{2+} release.

Fura-2 was used as a fluorescent Ca^{2+}-sensitive dye to measure $[Ca^{2+}]_i$ changes in the present study. NPC15199-induced Ca^{2+} entry in SCM1 cells was explored. The $[Ca^{2+}]_i$ rises were characterized, the concentration-response plots were established, and the pathways underlying Ca^{2+} entry and Ca^{2+} release were explored. The effect of NPC15199 on cell viability was also investigated.

Materials and Methods

Materials

The reagents for cell culture were from Gibco® (Gaithersburg, MD, USA). Aminopolycarboxylic acid/acetoxy methyl (fura-2/AM) and 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid/acetoxy methyl (BAPTA/AM) were from Molecular Probes® (Eugene, OR, USA). All other reagents were from Sigma-Aldrich® (St. Louis, MO, USA) unless otherwise indicated.

Cell Culture

SCM1 cells obtained from Bioresource Collection and Research Center (Taiwan) were cultured in F-12K medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin.

Solutions Used in $[Ca^{2+}]_i$ Measurements

Ca^{2+}-containing medium (pH 7.4) had 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 5 mM glucose. Ca^{2+}-free medium contained similar chemicals as Ca^{2+}-containing medium except that $CaCl_2$ was replaced with 0.3 mM ethylene glycol tetraacetic acid (EGTA) and 2 mM $MgCl_2$. NPC15199 was dissolved in ethanol as a 0.1 M stock solution. The other chemicals were dissolved in water, ethanol or dimethyl sulfoxide (DMSO). The concentration of organic solvents in the experimental solutions did not exceed 0.1%, and did not affect viability or basal $[Ca^{2+}]_i$.

$[Ca^{2+}]_i$ Measurements

The $[Ca^{2+}]_i$ was measured as previously described (6, 15). Confluent cells grown on 6 cm dishes were trypsinized and made into a suspension in culture medium at a density of 10^6 cells/ml. Cell viability was determined by trypan blue exclusion (adding 0.2% trypan blue to 0.1 ml cell suspension). Dead cells would stain blue. The viability was usually greater than 95% after the treatment. Cells were subsequently loaded with 2 μM fura-2/AM for 30 min at 25°C in the same medium. After loading, cells were washed with Ca^{2+}-containing medium twice and was made into a suspension in Ca^{2+}-containing medium at a density of 10^7 cells/ml. Fura-2 fluorescence measurements were performed in a water-jacketed cuvette (25°C) with continuous stirring; the cuvette contained 1 ml of medium and 0.5 million cells. Fluorescence was monitored with a Shimadzu RF-5301PC spectrofluorophotometer immediately after 0.1 ml cell suspension was added to 0.9 ml Ca^{2+}-containing or Ca^{2+}-free medium, by recording excitation signals at 340 nm and 380 nm and emission signal at 510 nm at 1-sec intervals. During the recording, reagents were added to the cuvette by pausing the recording for 2 sec to open and close the cuvette-containing chamber. For calibration of $[Ca^{2+}]_i$, after completion of the experiments, the detergent Triton X-100 (0.1%) and $CaCl_2$ (5 mM) were added to the cuvette to obtain the maximal fura-2 fluorescence. Then the Ca^{2+} chelator EGTA (10 mM) was added to chelate Ca^{2+} in the cuvette to obtain the minimal fura-2 fluorescence. Control experiments showed that cells bathed in a cuvette had a viability of 95% after 20 min of fluorescence measurements. $[Ca^{2+}]_i$ was calculated as previously described (10).

Cell Viability Assays

Viability was assessed as previously described (6, 15). The measurement of cell viability was based on the ability of cells to cleave tetrazolium salts by dehydrogenases. Augmentation in the amount of developed color directly correlated with the number of live cells. Assays were performed according to manufacturer's instructions specifically designed for this assay (Roche Molecular Biochemical, Indianapolis, IN, USA). Cells were seeded in 96-well plates at a density of 10,000 cells/well in culture medium for 24 h in the presence of NPC15199. The cell viability detecting reagent 4-[3-[4-Iodophenyl]-2-4(4-nitrophenyl)-2H-5-tetrazolio-1,3-benzene disulfonate] (WST-1; 10 μl pure solution) was added to samples after NPC15199 treatment, and cells were incubated for 30 min in a humidified atmosphere. In experiments using BAPTA/AM to chelate cytosolic Ca^{2+}, cells were treated with 5 μM BAPTA/AM for 1 h prior to incubation with NPC15199. The cells were washed once with Ca^{2+}-containing medium and incubated with/without NPC15199 for 24 h. The absorbance of samples (A_{450}) was determined using an enzyme-linked immunosorbent assay (ELISA) reader. Absolute optical density was normalized to the absorbance of unstimulated cells in each plate and expressed as a percentage of the control value.

Statistics

Data are reported as mean ± SEM of three experiments. Data were analyzed by one-way analysis of variances (ANOVA) using the Statistical Analysis System (SAS®, SAS Institute Inc., Cary, NC, USA). Multiple comparisons between group means were performed by *post-hoc* analysis using the Tukey's HSD (honestly significant difference) procedure. A *P*-value less than 0.05 was considered significant.

Results

Effect of NPC15199 on $[Ca^{2+}]_i$

The effect of NPC15199 on basal $[Ca^{2+}]_i$ was examined. Fig. 1A shows that the basal $[Ca^{2+}]_i$ was 51 ± 1 nM. At concentrations between 400-1000 μM, NPC15199 evoked $[Ca^{2+}]_i$ rises in a concentration-dependent fashion in Ca^{2+}-containing medium. At 1000 μM, NPC15199 evoked $[Ca^{2+}]_i$ rises that reached a net increase of 95 ± 5 nM (n = 3) followed by a decay. The $[Ca^{2+}]_i$ signal saturated at 1000 μM NPC 15199 because 1500 μM NPC15199 did not evoke a larger response. Fig. 1B shows that in Ca^{2+}-free medium, 1000 μM NPC15199 evoked $[Ca^{2+}]_i$ rises of 50 ± 2 nM. 600-1000 μM NPC15199 induced concentration-dependent rises in $[Ca^{2+}]_i$. Fig. 1C shows the concentration-response plot of NPC 15199-induced response. The EC_{50} value was 453 ±

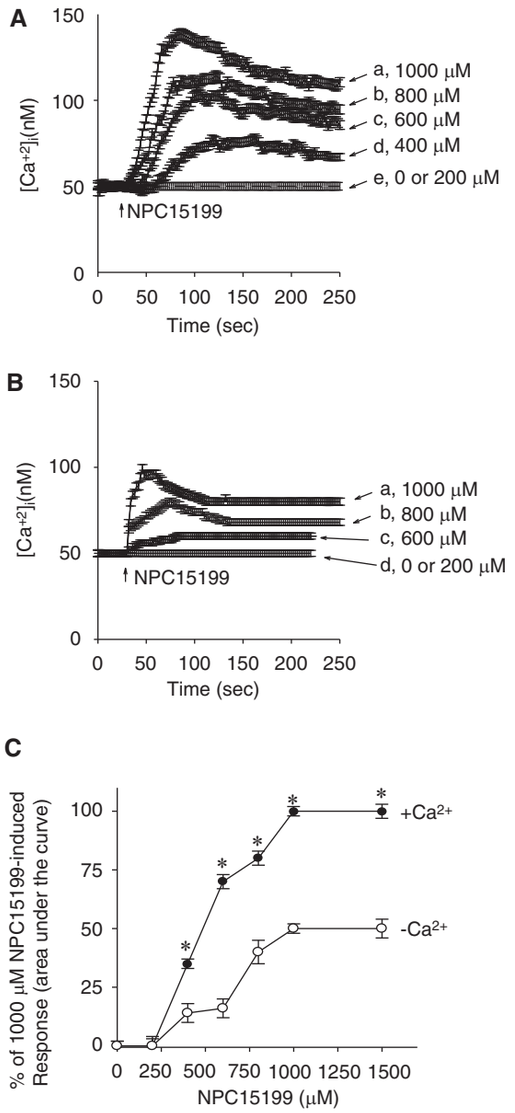

Fig. 1. Effect of NPC15199 on $[Ca^{2+}]_i$ in SCM1 cells. (A) NPC 15199-induced $[Ca^{2+}]_i$ rises in fura-2-loaded cells. NPC 15199 was added at 25 sec. The concentration of NPC 15199 was indicated. The experiments were performed in Ca^{2+}-containing medium. (B) Effect of NPC15199 on $[Ca^{2+}]_i$ in Ca^{2+}-free medium. NPC15199 was added at 25 sec in Ca^{2+}-free medium. (C) Concentration-response plots of NPC15199-induced $[Ca^{2+}]_i$ rises in the presence or absence of extracellular Ca^{2+}. Y axis is the percentage of the net (baseline subtracted) area under the curve (25-250 sec) of the $[Ca^{2+}]_i$ rises induced by 1000 μM NPC15199 in Ca^{2+}-containing medium. Data are mean ± SEM of three separate experiments. *$P < 0.05$ compared to open circles.

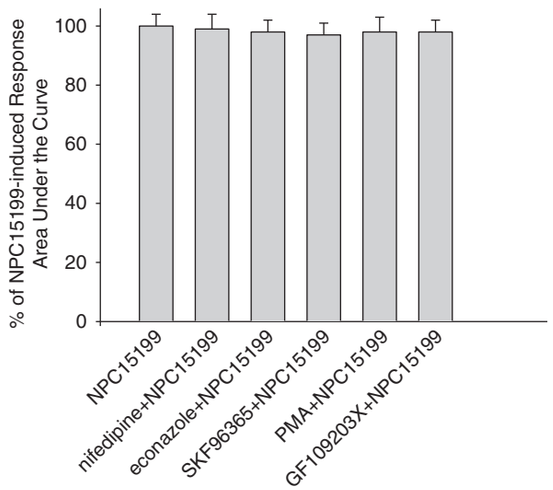

Fig. 2. Lack of an effect of Ca^{2+} channel modulators on NPC 15199-induced $[Ca^{2+}]_i$ rises. In blocker- or modulator-treated groups, the reagent was added 1 min before NPC 15199 (1000 μM). The concentration was 1 μM for nifedipine, 0.5 μM for econazole, 5 μM for SKF96365, 10 nM for phorbol 12-myristate 13-acetate (PMA), and 2 μM for GF109203X. Data are expressed as the percentage of control (1st column) that is the area under the curve (25-200 sec) of 1000 μM NPC15199-induced $[Ca^{2+}]_i$ rises in Ca^{2+}-containing medium, and are mean ± SEM of three separate experiments.

12 μM or 650 ± 13 μM in the presence or absence of extracellular Ca^{2+}, respectively, by fitting to a Hill equation.

Pathways of NPC15199-Induced Ca^{2+} Entry

Fig. 1 shows that NPC15199-induced Ca^{2+} response saturated at 1000 μM; thus in the following experiments the response induced by 1000 μM NPC15199 was used as control. The effect of EC_{50} value (453 μM in Ca^{2+}-containing medium or 650 μM in Ca^{2+}-free medium, respectively) of NPC15199 on Ca^{2+} response was also investigated. Three Ca^{2+} entry inhibitors: nifedipine (1 μM), econazole (0.5 μM) and SKF96365 (5 μM); phorbol 12-myristate 13 acetate (PMA; 1 nM; a protein kinase C (PKC) activator); and GF109203X (2 μM; a PKC inhibitor) were applied 1 min before NPC15199. These compounds did not inhibit 1000 μM NPC15199-evoked $[Ca^{2+}]_i$ rises (Fig. 2). Consistently, These compounds did not prevent 453 μM NPC15199-evoked $[Ca^{2+}]_i$ rises (data not shown).

Sources of NPC15199-Induced Ca^{2+} Release

The endoplasmic reticulum was shown to be a dominant Ca^{2+} store in most cell types including SCM1 human gastric cancer cells (6, 7, 15). Thus the role of the endoplasmic reticulum in NPC15199-evoked Ca^{2+} release in SCM1 cells was explored. To exclude the contribution of Ca^{2+} entry, the experiments were performed in Ca^{2+}-free medium. Fig. 3A shows that addition of 50 μM 2,5-di-tert-butylhydroquinone (BHQ), an endoplasmic reticulum Ca^{2+} pump inhibitor (28), induced $[Ca^{2+}]_i$ rises of 51 ± 3 μM. Subsequently added 1000 μM NPC15199 induced $[Ca^{2+}]_i$ rises of 15 ± 3 nM. Fig. 3B shows that after NPC15199-induced $[Ca^{2+}]_i$ rises had decayed to a plateau, addition of BHQ did not induce $[Ca^{2+}]_i$ rises. Thapsigargin (1 μM), another endoplasmic reticulum Ca^{2+} pump inhibitor (25), was applied to confirm BHQ's data. Fig. 3C shows that thapsigargin induced $[Ca^{2+}]_i$ rises of 60 ± 2 nM. NPC15199 (1000 μM) added at 500 sec induced $[Ca^{2+}]_i$ rises of 6 ± 1 nM. Conversely, Fig. 3D shows that after NPC15199 treatment, addition of thapsiargin at 500 sec induced $[Ca^{2+}]_i$ rises of 4 ± 1 nM. The effect of 650 μM NPC15199 on Ca^{2+} release was consistent with the results of using 1000 μM NPC15199 (data not shown).

NPC15199 Induced $[Ca^{2+}]_i$ Rises via a PLC Independent Pathway

PLC is a pivotal enzyme that modulates the release of Ca^{2+} from the endoplasmic reticulum (2, 8, 9). Because NPC15199 released Ca^{2+} from the endoplasmic reticulum, the role of PLC in this process was explored. U73122 (26), a PLC inhibitor, was applied to explore if the activation of this enzyme was required for NPC15199-induced Ca^{2+} release. Fig. 4A shows that ATP (10 μM) induced $[Ca^{2+}]_i$ rises of 65 ± 2 nM. It has been shown that ATP is a PLC-dependent agonist of $[Ca^{2+}]_i$ rises in gastric cell types (3, 4). Purinergic receptors were specific classes of membrane receptors that mediate relaxation of gut smooth muscle as a response to the release of ATP (P2 receptors) or adenosine (P1 receptors) (3, 4). P2 receptors have further been divided into five subclasses: P2X, P2Y, P2Z, P2U, and P2T. P2Y receptors included $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$. P2Y receptors are present in almost all human tissues where they exert various biological functions based on their G-protein coupling (3, 4). It has been shown that $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ are present in gastric cancer cells (3, 4). Therefore, it seems that SCM1 cells may express at lease one type of P2Y receptors like $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$.

Fig. 4B shows that incubation with 2 μM U73122 did not change basal $[Ca^{2+}]_i$ but abolished ATP-induced $[Ca^{2+}]_i$ rises. This suggests that U73122 effectively suppressed PLC activity. Fig. 4B also shows that incubation with 2 μM U73122

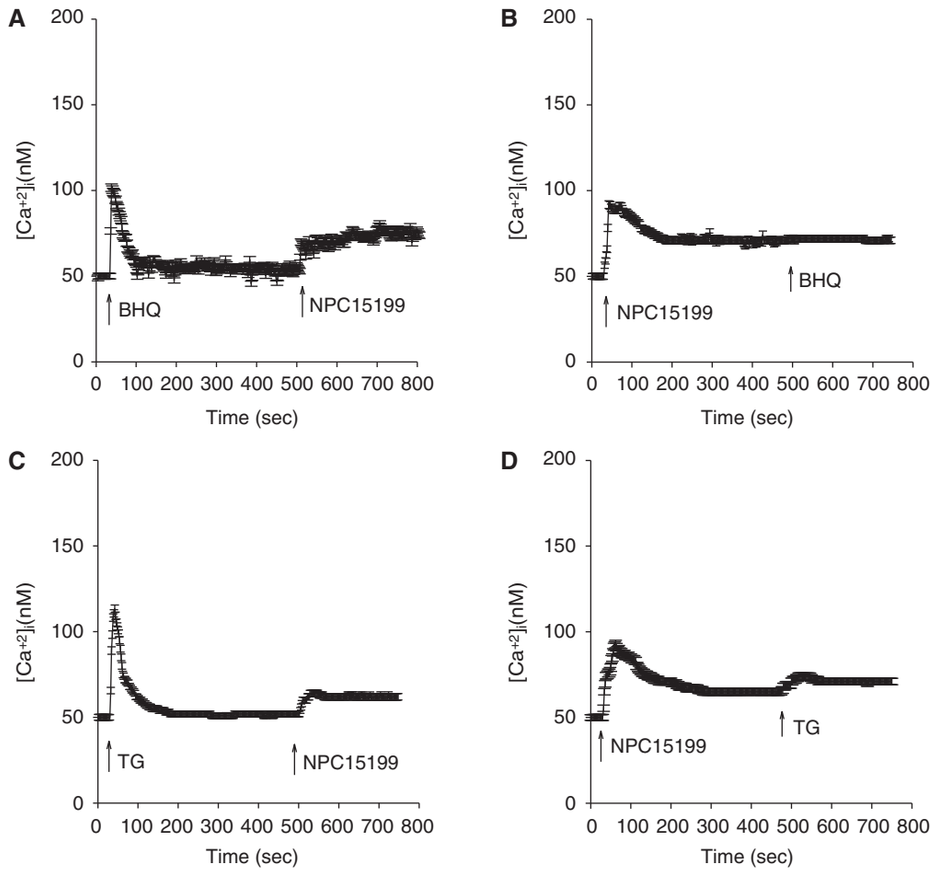

Fig. 3. Effect of BHQ and thapsigargin on NPC15199-induced Ca^{2+} release. (A)-(D) BHQ (50 μM), thapsigargin (1 μM) and NPC15199 (1000 μM) were added at time points indicated. Experiments were performed in Ca^{2+}-free medium. Data are mean ± SEM of three separate experiments.

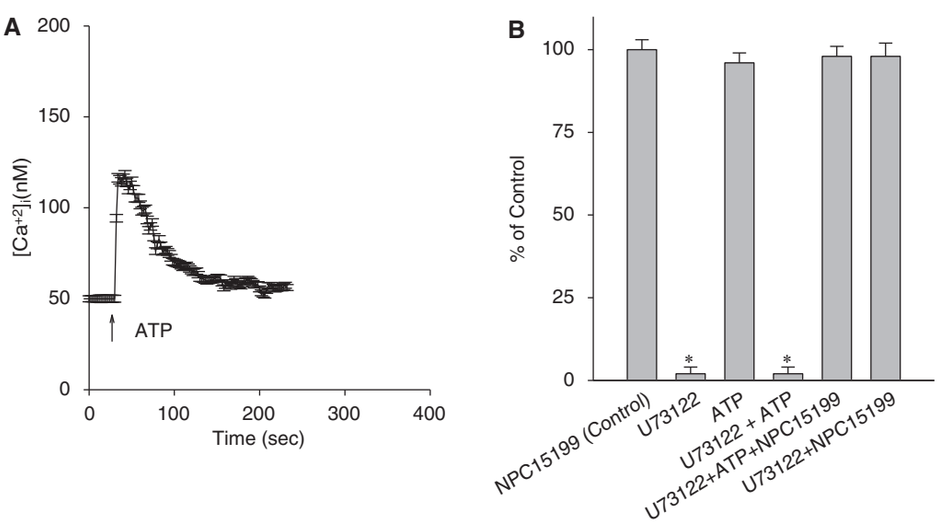

Fig. 4. Effect of U73122 on NPC15199-induced Ca^{2+} release. Experiments were performed in Ca^{2+}-free medium. (A) ATP (10 μM) was added as indicated. (B) U73122 (2 μM), ATP, and NPC15199 (1000 μM) were added as indicated. Data are mean ± SEM of three separate experiments. *$P < 0.05$ compared to first bar (control). Control is the area under the curve of 1000 μM NPC15199-induced $[Ca^{2+}]_i$ rises (25-250 sec).

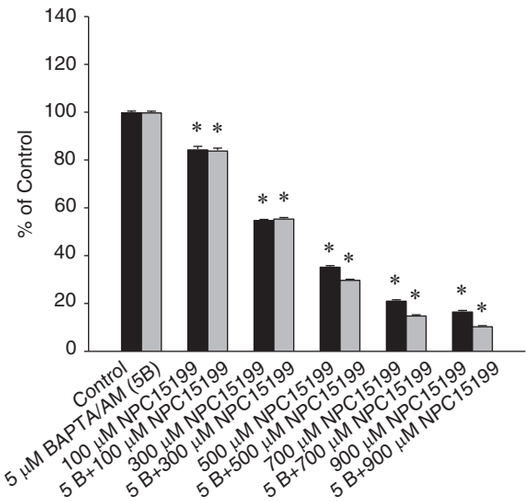

Fig. 5. Effect of combination of BAPTA/AM and NPC15199 on viability of SCM1 cells. In experiments using BAPTA/AM to chelate cytosolic Ca^{2+}, cells were treated with 5 μM BAPTA/AM for 1 h prior to incubation with NPC 15199. The cells were washed once with Ca^{2+}-containing medium and incubated with/without NPC15199 (0-900 μM) for 24 h, and then cell viability assay was performed. Data are mean ± SEM of three separate experiments. Each treatment had six replicates (wells). Data are expressed as percentage of control that is the increase in cell numbers in NPC15199-free groups. Control had 10,657 ± 714 cells/well before experiments, and had 13,221 ± 745 cells/well after incubation for 24 h. In each group, the Ca^{2+} chelator BAPTA/AM (5 μM) was added to cells followed by treatment with NPC15199 in Ca^{2+}-containing medium. Cell viability assay was subsequently performed. *$P < 0.05$ compared to control.

did not alter basal $[Ca^{2+}]_i$ or 1000 μM NPC15199-induced $[Ca^{2+}]_i$ rises. U73343 (2 μM), a U73122 analogue, failed to have an inhibition on ATP-induced Ca^{2+} signal (not shown). Furthermore, the effect of 650 μM NPC15199 on PLC pathway was consistent with the results of using 1000 μM NPC 15199 (data not shown).

Effect of NPC15199 on Cell Viability

Experiments were performed to examine the effect of NPC15199 on viability of SCM1 cells. Cells were treated with 0-900 μM NPC15199 for 24 h, and the tetrazolium assay was performed. In the presence of 100-900 μM NPC15199, cell viability decreased in a concentration-dependent manner (Fig. 5).

Relationship between NPC15199-Induced $[Ca^{2+}]_i$ Rises and Cell Death

The next issue was whether NPC15199-induced cell death was caused by preceding $[Ca^{2+}]_i$ rises. The intracellular Ca^{2+} chelator BAPTA/AM (27) was used to prevent $[Ca^{2+}]_i$ rises during NPC15199 treatment. Fig. 5 also shows that 5 μM BAPTA/AM loading did not alter the control value of cell viability. NPC 15199 (100-900 μM) did not induce $[Ca^{2+}]_i$ rises in BAPTA/AM-treated cells (not shown). BAPTA/AM loading did not reverse NPC15199-induced cell death.

Discussion

N-(fluorenyl-9-methoxycarbonyl) amino acids are a class of anti-inflammatory agents including NPC14686, NPC14688, NPC14692, NPC15199, NPC15533, NPC15521, NPC15533, NPC15573, NPC15667, NPC15699, and NPC15895. Their differences exist in the modulation of amino acids. For example, NPC14686 is L-homophenylalanine, NPC 14688 is L-alanine, NPC14692 is glycine, NPC15199 is L-leucine, NPC15521 is N-(fluore-nyl-9-ethoxy-carbonyl)-L-leu-cine, NPC15533 is DL-1,2,3,4-tetrahydroisoquinoline, NPC15573 is L-tert-leucine, NPC15667 is L-norleucine, NPC15669 is N-[9H-{2,7-dimethylfluorenyl-9-methoxy}carbonyl]-L-leucine, NPC15895 is N-[9H-{3-fluorenyl-9-proprionyl}]-L-homophenylala-nine. These agents possess different activities in a broad range of animal models of inflammation (5, 17).

NPC15199, a peroxisome proliferator-activated receptor gamma (PPAR gamma) agonist, participated in anti-inflammation in different models (5, 16, 17, 21). It has been shown that NPC15199 induced other physiological actions such as anti-tumor effect (1, 18). Furthermore, several reports showed that NPC15199 induced $[Ca^{2+}]_i$ rises in BFTC cells (13) and MDCK cells (12). Our study shows that NPC15199 also increased $[Ca^{2+}]_i$ in SCM1 human gastric cancer cells. NPC15199 increased $[Ca^{2+}]_i$ in SCM1 cells by depleting Ca^{2+} stores and causing Ca^{2+} influx. The results suggest that NPC15199 induced Ca^{2+} entry because NPC15199-induced Ca^{2+} signal was inhibited by removal of extracellular Ca^{2+}.

The mechanism of NPC15199-induced Ca^{2+} entry was examined. Store-operated Ca^{2+} channels have been shown to play a role in stimulants-induced $[Ca^{2+}]_i$ rises in SCM1 cells (7). Because NPC15199-evoked $[Ca^{2+}]_i$ rises were not inhibited by nifedipine, econazole and SKF96365, the results suggest that NPC 15199 might not cause Ca^{2+} entry *via* store-operated Ca^{2+} entry. These three chemicals have been used as blockers of store-operated Ca^{2+} entry in many cell models (11, 14, 20, 22). However, there are so far no selective inhibitors for this channel. The activity of many protein kinases is known to associate with Ca^{2+} homeostasis (2, 9). Our data show that NPC15199-evoked $[Ca^{2+}]_i$ rises were not altered when PKC activ-

ity was activated or inhibited. Therefore, it suggests that PKC may not involve in NPC15199-induced Ca^{2+} entry. The pathways that are responsible for NPC 15199-induced Ca^{2+} entry remains to be explored.

Regarding the Ca^{2+} stores involved in NPC15199-evoked Ca^{2+} release, the BHQ/thapsigargin-sensitive endoplasmic reticulum stores seem to be the dominant one. However, because BHQ/thapsigargin did not completely inhibited NPC15199-induced Ca^{2+} release, NPC15199 might also release Ca^{2+} from other minor stores such as mitochondria, nuclei, *etc.* (8, 9). In terms of the role of PLC in NPC15199-induced Ca^{2+} release, the PLC inhibitor U73122 did not inhibit NPC15199-induced Ca^{2+} release in MDCK cells (12) and SCM1 cells used in our study. Therefore, the data suggest that the Ca^{2+} release was *via* a PLC-independent mechanism. Previous evidence showed that phospholipase A_2/reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase may collaborate to regulate Ca^{2+} influx and Ca^{2+} release (24, 29). Therefore, it appears that phospholipase A_2/NADPH oxidase may involve in NPC15199-evoked release.

In previous studies, NPC15199 at concentrations of 200-1000 μM induced concentration-dependent $[Ca^{2+}]_i$ rises in BFTC cells (13). NPC15199 (200-1000 μM) induced $[Ca^{2+}]_i$ rises in MDCK cells in a concentration-dependent fashion (12). Similarly, NPC15199 (400-1000 μM) induced concentration-dependent $[Ca^{2+}]_i$ rises in SCM1 cells. Furthermore, NPC15199 induced Ca^{2+} release from the endoplasmic reticulum and activated Ca^{2+} entry in these three cell types.

Cell death may associate or dissociate with preceding $[Ca^{2+}]_i$ rises, depending on cell type and the agonist (19, 23). Our findings show that NPC15199 (100-900 μM)-induced cell death was independent of Ca^{2+}. Therefore, it suggests that NPC15199-induced cell death was dissociated from $[Ca^{2+}]_i$ rises. NPC15199 has not been tested in patients, thus the achievable plasma level is unknown. However, previous studies were performed to explore the plasma level of NPC15199 in animals. The plasma level of NPC15199 may reach 20-30 μM (1, 18). This level may be expected to go much higher in animals with liver or kidney disorders. Our data show that NPC15199 at a concentration of 100 μM induced slight cell death. Therefore, our data may be clinically relevant.

In sum, the results show that NPC15199 induced Ca^{2+} release from endoplasmic reticulum in a PLC-independent manner and also caused Ca^{2+} influx *via* PKC-insensitive non-store-operated Ca^{2+} entry in SCM1 human gastric cancer cells. Our data also show that NPC15199-induced cell death is not caused by preceding rises in $[Ca^{2+}]_i$. The effect of NPC15199 on Ca^{2+} movement in human gastric cancer cells should be considered in other types of *in vitro* research.

Acknowledgments

This work was supported by the grant RD102013. The authors declare no conflicts of interest.

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