Polyamine Induces Apoptosis Through the Calcium Signaling in Human Prostate Cancer Cells

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Polyamines are essential for the normal cell growth and differentiation. They are also known to have paradoxical dual effects on cell proliferation. In this paper we show that the excess amount of polyamines induces apoptosis through the modulation of calcium signaling in LNCaP human prostate cancer cells. Polyamines, particularly spermidine and spermine, stimulated cell proliferation at a lower concentration (under 10 µM), but it inhibited cell viability at a higher concentration (40 µM). The levels of intracellular Ca^{2+} concentration were increased only at a high concentration of polyamines treatment without any noticeable changes at lower concentrations. Nifedipine did not alter the increase of polyamine-induced Ca^{2+} levels, but flufenamic acid totally abolished the increase of intracellular Ca^{2+} levels. These results mean that polyamines induce Ca^{2+} influx from the surroundings through nonselective cation channels on the cell membrane. The expression of Bcl-2 protein was almost completely blocked, but the level of Bax protein was increased dramatically in the cells treated with high concentration of polyamine. The present study shows that polyamines at a high concentration induce apoptosis through the modulation of intracellular calcium signaling. The increase of intracellular calcium level induced by polyamines, was possibly a result from the extracellular calcium influx through the nonselective cation channels.

Key words – Apoptosis, Bcl-2, Bax, calcium, polyamines

Introduction

The polyamines including putrescine, spermidine and spermine are essential for cell growth and differentiation. Because of their polycationic nature and unique charge distribution, polyamines are also believed to be important in DNA stabilization, RNA and protein synthesis, membrane stabilization and transport, and receptor responses[12,22]. Cell proliferation and a high rate of transformation induced by growth factors are characterized by the increased polyamine biosynthesis and an enhanced uptake of polyamines. In general, rapidly growing cells or tumor cells have a higher level of polyamine than slowly growing, normal or quiescent cells. Polyamine concentrations may vary during the cell cycle and increased levels have been found after tropic stimuli and is also associated with the development of proliferate diseases such as cancer[22].

However, polyamines have paradoxical dual roles in inducing apoptosis and in its prevention. The prostate has one of the highest polyamine concentrations of any tissue. Normal and benign hyperplastic prostatic tissues have a high content of spermine whereas in tumor tissue, especially in prostatic carcinoma with metastases, spermine levels are reduced. A strong decrease in the concentration of spermine in the prostate could indicate a conversion of prostatic cells from a benign into a malignant phenotype [16]. The spermine can negatively regulate the growth of prostatic cancer cells and may explain the slow rate of primary tumor expansion in the prostate. It has been reported that spermine inhibits the growth of prostatic carcinoma *in vitro* as well as *in vivo*[15].

Even though the precise role of polyamines in apoptosis appears to be complex and not clear, many papers suggest the involvement of polyamines in the activation of caspases[10,20,21] and calcium modulation[2,10,23] in the apoptotic processes. Caspases, a family of cysteine protease, play a central role in the signaling and execution of apoptosis[19,20]. The activation of caspase-3 during apoptosis is regulated by multiple pathways. One of the important pathways to activate caspase-3 is mediated by the alteration of mitochondria. Mitochondria depolarization and/or caspase-3 activation are known to be regulated by

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the cytosolic free calcium concentration in many cell systems[1,18]. Mitochondria are involved in multiple Ca^{2+} transport mechanisms. When cellular Ca^{2+} is overloaded, mitochondria take up cytosolic free Ca^{2+}, which induces the mitochondria membrane potential change. The collapse of the membrane potential results in the release of cytochrome c and other apoptosis-inducing factors from mitochondria, which is followed by the activation of caspase, nuclear fragmentation and cell death. Members of the Bcl-2 family are also important as anti-apoptotic or pro-apoptotic proteins in apoptosis regulation. These proteins appear to differently regulate intracellular Ca^{2+} level[13].

Ca^{2+} signaling is responsible for the regulation of most processes in normal cells. Thus, it is not surprising that changes in the cytosolic free Ca^{2+} concentration as well as in sub-cellular organelles have a causal role in cell growth and death induced by various stimuli. The elevation of intracellular Ca^{2+} level activates a wide variety of Ca^{2+}-sensitive enzymes. These enzymes may generate signaling molecules for the recruitment of mitochondria to the apoptotic cascade or for the activation of caspase enzymes. Calpains (cysteine proteases), calcineurin (serine/ threonine protein phosphatase), protein kinase C (PKC), and caspases are the cytoplasmic targets of Ca^{2+}, which is directly connected to the executioners of apoptosis[6]. Increased intracellular Ca^{2+} also activates Ca^{2+}/Mg^{2+}-dependent endonuclease, resulting in DNA fragmentation, which is the most characteristic biochemical feature of apoptosis.

Since the prostate tissue has a high concentration of polyamine, it is worth it to study the roles of polyamine in the proliferation of prostatic cells. In the present paper, we show that polyamines induce apoptosis through the mobilization of intracellular calcium in the LNCaP human prostate cancer cells. To investigate the influence of polyamine, cell viability was checked in the presence of various concentrations of putrescine, spermidine, and spermine. The level change in cytosolic free Ca^{2+} was measured in the cells incubated in the media with or without calcium. Furthermore, the expression of antiapoptotic protein, Bcl-2, and proapoptotic protein, Bax, were checked during the polyamine-induced apoptosis.

Materials and Methods

Chemicals and cell culture

Putrescine (tetracethylenediamine), spermidine (N-[3-aminopropyl]-1,4-butanediamine), spermine (N,N'-bis[3-aminopropyl]-1,4-butanediamine), 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl I)-dimethyl ester (Nifedipine), 2-{[3-(trifluoro methyl) phenyl] amino} benzoic acid (Flufenamic acid), 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Fluo-3/AM (acetomethoxy ester), pluronic F-127, propidium iodide (PI), Hoechst 33342, ribonuclease A, dimethyl sulfoxide (DMSO), and Dulbecco's modified Eagle's medium (DMEM, with L-glutamine and 1,000 mg/L Glucose) were purchased from Sigma Chemical Co (St. Loues, MO, USA). Fetal bovine serum (FBS) was purchased from GIBCO (New York, USA). All other chemicals were purchased from standard commercial sources. LNCaP prostate carcinoma cell line was obtained from the Korean Cell Line Bank (Seoul, Korea), and maintained in DMEM containing phenol red with 10,000 units/ml penicillin G, 10 mg/ml streptomycin, and 5% heat-inactivate FBS in a humidified atmosphere of 95% air; 5% CO_2 at 37°C. Culture media were changed every 2 or 3 days. Cells were harvested using trypsin-EDTA and then subcultured at weekly intervals.

Measurement of cell viability

The cell viability was determined by MTT assay. A detailed description of the assay is given elsewhere[8]. Briefly, cells were washed twice with cold phosphate-buffered saline (PBS) after appropriate treatment periods, then incubated in a culture medium with 0.5 mg/ml MTT dye for 4 hr. After incubation, the medium was removed and the converted dye was solublized in a 1:1 mixture of DMSO and EtOH. The color intensity was measured by the ELISA Reader (Hitach, Japan) with 540 nm filter.

Measurements of intracellular calcium

The level of cytosolic free Ca^{2+} concentration in LNCaP cells was measured using a fluorometric plate reader (Tecan, Germany). The cells grown in DMEM were washed twice and resuspened in HBSS (120 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 2 nmM $MgCl_2$, 10 mM HEPES, and 12 mM glucose). For Ca^{2+}-free HBSS, $CaCl_2$ was removed and 0.5 mM EGTA was added. For fluo-3/AM loading, $2x10^6$ cells were resuspended in Ca^{2+}-free HBSS containing 5 µM fluo-3/AM and 0.02% pluronic F-127. Incubation was performed in the dark at 37°C for 30 min. Cells were then washed with Ca^{2+}-free HBSS and split into 48 multi well plates at $2.5x10^5$ cells/well and treated with 2 mM calcium,

polyamines and two kinds of Ca^{2+} blockers. The intensity of fluorescence was measured with 485 nm excitation and 535 nm emission.

Assessment of morphology

Nuclear condensation and/or chromatin fragmentation found during early apoptosis was determined morphologically by the confocal laser scanning microscopy (CLSM) (Carl Zeiss, Oberkohen, Germany) after labeling PI and Hoechst dye. The cells with each treatment were harvested onto a clean, fat-free glass slide with cellspin (Hanil Sci Industrial, Korea). Then, the cells were fixed for 30 min in 4% paraformaldehyde and then stained in 4 µg/ml Hoechst 33342 or in 10 µg/ml PI with 50 µg/ml ribonuclease A for 1 h at 37°C. The samples were observed and photographed under a CLSM.

Western blot analysis

Cells were collected by centrifugation at 5,000 rpm for 10 min at 4°C. Proteins were extracted by washing cells twice with ice-cold PBS and incubating them for 60 min on ice in lysis buffer containing 50 mM Tris-HCl (pH 7.6), 300 mM NaCl, 0.5% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 2 µg/ml aprotinin, and 2 µg/ml leupeptin. For Western blotting, an aliquot of 60 µg of protein was separated by sodium dodecylsulfate-polyacrylamid gel electrophoresis (SDS-PAGE) and then transferred to a nitrocellulose transfer membrane. Blotting was performed using the primary antibodies of anti-Bcl-2 (1:1000 dilution) and anti-Bax (1:1000 dilution), and then with the proper secondary antibody (Sigma, MO, USA). The immuncomplexes were detected by using the ECL (enhanced chemiluminescence) detection kit (Amersham Pharmacia Biotech Korea, Inc., Seoul, Korea).

Statistical analysis

All experiments were carried out at least in triplicate. Results were expressed as mean ± SEM. . Statistical significances between the control and the treatment group were determined by one-way analysis of variance and then followed by the Student T test. In all cases, a p value less than 0.05 was considered statistically significant.

Results

Measurement of cell viability

In order to determine the effects of polyamines on the

LNCaP cell growth, cells were treated for 4 days with each polyamine (0.1-20 mM putrescine, 1-40 µM spermidine or spermine). As shown in Fig. 1, putrescine, even at much higher concentration compared to the concentrations of spermidine and spermine, had no significant effect on the cell growth throughout the concentration range tested. However, spermidine and spermine showed dual effects on the cell growth according to the concentration of each polyamine externally added. In spermidine treatment, cell viability were noticeably increased at lower concentration. At day 3 of spermidine treatment, cell viability was increased to 145% and 130% of the control at 1 and 10 µM, respectively. At even a longer incubation period, the cell growth was not altered by the spermidine treatment at a concentration under 10 µM (data no shown). However, at higher concentration of spermidine, cell viability was severely reduced. Spermine also stimulated the cell growth at lower concentration. However, like spermidine, spermine at 20 and 40 µM inhibited the cell growth to 33% and 12% of the control, respectively.
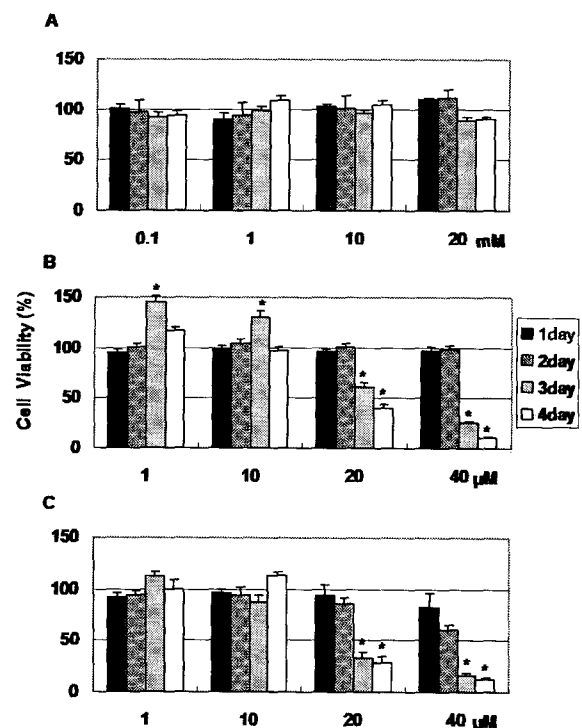

Fig. 1. Effect of polyamines on the cell viability. At day 0, cells were plated at the density of 5×10^4 cells/ml in 48 multi-well dish. The cell viability was measured by MTT assay at day intervals. (A) putrescine, (B) spermidine, (C) spermine. Data represent the mean ± SD for at least 3 separate experiments. (*p<0.05 vs. control).

Influence of polyamine on a intracellular calcium level

As shown in Fig. 2, there was a significant increase in the levels of intracellular Ca^{2+} in LNCaP cells after the treatment of polyamines in the medium containing 2 mM calcium. The fluorescence intensity was increased in a time- dependent manner within 5 min. A longer incubation up to 30 min did not accentuate this effect throughout the polyamine concentration range tested. Compared to putrescine, spermidine and spermine induced much higher level of intracellular calcium. The fluorescence intensity was increased to a maximum 119% of the control at 20 mM putrescien (Fig. 2A). However, the levels of intracellular calcium concentration were increased up to 133% and 137% of the control within 5min with
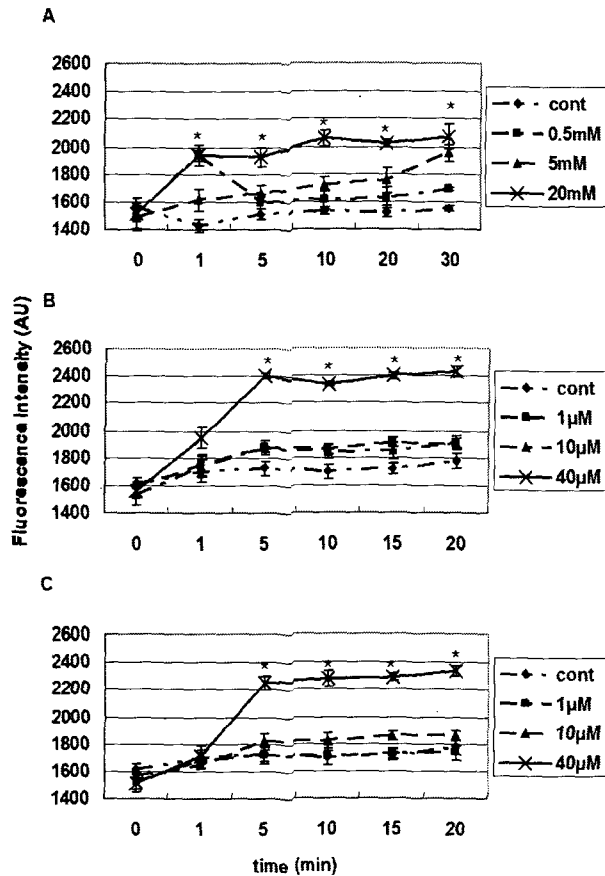

A

B

Fluorescence Intensity (AU)

C

time (min)

Fig. 2. Polyamine-induced intracellular Ca^{2+} increase in a calcium medium. For the measurement of intracellular Ca^{2+} level, cells were loaded with fluo-3/AM and plated in 48 multi-well at the density of 2.5×10^5 cells/well for each treatment (see M&M in detail). (A) putrescine, (B) spermidine, (C) spermine. (*p<0.03 vs. control)

spermidine and spermine at each 40 μM, respectively (Fig. 2B, C). These results coincided with the previous finding that putrescine treatment did not noticeably influence the cell viability compared to the significant influence of spermidine and spermine (Fig. 1). The levels of intracellular calcium concentration were not changed at all at a lower spermidine and spermine concentration. These results might be related to the cell growth accelerating ability of spermidine and spermine at a lower concentration. The increase of intracellular Ca^{2+} level with the polyamine treatment might be a result from the influx of extracellular calcium and/or the release of the stored calcium. To exclude the extracellular calcium influx, the level change of intracellular Ca^{2+} was measured in the Ca^{2+}-free medium. In all three polyamine treatments, the increase of fluorescence intensity was negligible compared to the increase in the Ca^{2+} buffer (Fig. 3). These results suggest that polyamine induced the

A

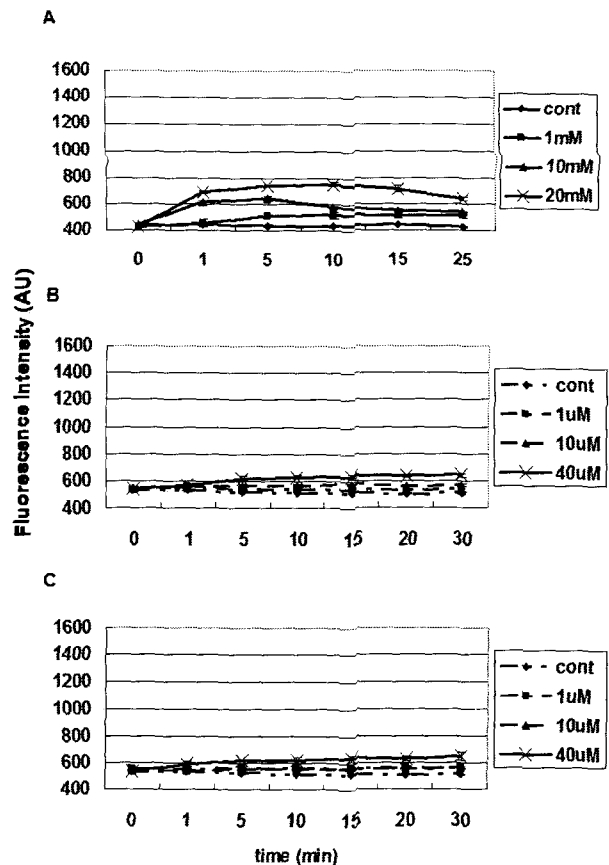Fluorescence Intensity (AU)

B

C

time (min)

Fig. 3. Polyamine-induced intracellular Ca^{2+} increase in a calcium-free medium. Cells were treated as the same way as in Fig 2. (A) putrescien, (B) spermidine, (C) spermine. (*p<0.03 vs. control)

increase of cytosolic free Ca^{2+} by the extracellular calcium influx through the cell membrane.

Intrecellular calcium level change in the presence of calcium blockers

To find which type of membrane Ca^{2+} channels mediates the polyamine-induced Ca^{2+} influx, two kinds of Ca^{2+} channel blockers were used. As shown in Fig. 4, 100 µM nifedipine, the voltage-sensitive Ca^{2+} channel blocker , did not affect the spermine-induced Ca2+ influx, whereas flufenamic acid at 100 µM, the nonselective cation channel (NSCC) blocker, completely blocked the spermine-induced Ca^{2+} influx. The spermidine-induced Ca^{2+} increase was also blocked in the same manner by flufenamic acid but not by nifedifine (data not shown). These results suggest that polyamine induces Ca^{2+} influx through the activation of NSCC in the cell membrane.

Assessment of cell morphology

The cells were treated with spermine, and the morphological change of nuclei was observed by a laser confocal scanning microscope after dye treatments. As shown in Fig. 5, the nuclei of the cells treated with 10 µM spermine for 3 days were the same as those in the untreated control in both Hoechst and PI staining. However, the nuclei in the cells treated with 40 µM spermine were severely condensed as shown in PI staining (Fig. 5A). In Hoechst staining, condensed and fragmented chromatin were found as

Fig. 5. Propidium iodide (A) and Hoechst (B) stained nuclei morphology in spermine-induced apoptosis. Cells were treated with spermine for 3days. After fixation and staining at each dye, cells were observed under the confocal laser scanning microscope. A representative of 3 experiments is shown.

bright spots in the cells treated with 40 µM spermine which is a typical early apoptotic morphology. The similar results were also found in the cells treated with each 10 and 40 µM spermidine (data not shown). These results coincided the previous findings that polyamine at only a higher concentration inhibited cell proliferation and induced an increase of intracellular Ca^{2+} level.

Effects of spm on Bcl-2 and Bax protein expression.

Members of the Bcl-2 family dually regulate the apoptotic process. Bcl-2 is known to negatively regulate it and Bax positively regulates it. The changes in those two protein levels were analyzed by Western blotting. As shown in Fig 6, Bcl-2 expression was similar to the control in the cells treated with 10 µM spermine for 3day. However with 40 µM spermine, Bcl-2 was not detected at all. In the experiment of Bax expression, the opposite results were
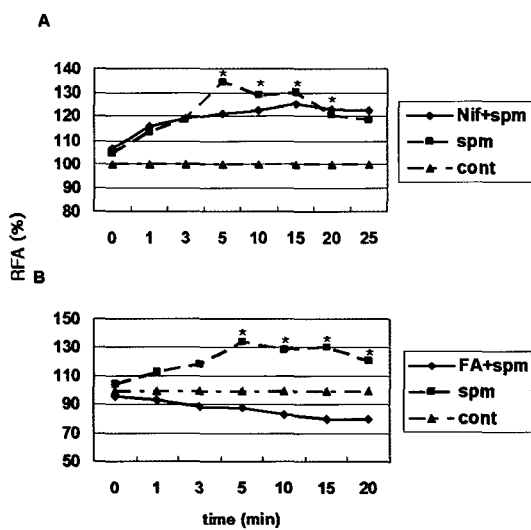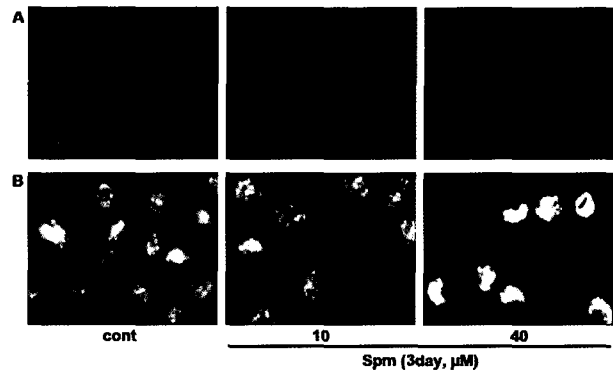

Fig. 4. Effect of Ca^{2+} channel blockers in spermine-induced Ca^{2+} increase. Cells were treated as the same procedure as in Fig. 2. RFA; relative fluorescence activity. (A) nifedipine, (B) flufenamic acid. (*p<0.05 vs. control).
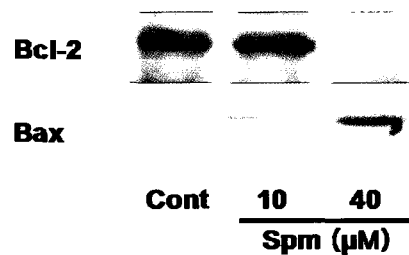

Fig. 6. Western blots showing the effects of spermine on the Bcl-2 and Bax expression.
Cells were treated with spermine for 3 days. After protein separation by SDS-PAGE, the proteins were transferred to nitrocellulose membrane and detected by using the ECL detection method. A representative of 3 experiments is shown.

found. In 10 μM spermine treatment, the expression of Bax was very low, but its expression was very prominent in 40 μM spermine treatment. Therefore, it is proven very clearly that excess polyamine treatment blocked the expression of Bcl-2 but stimulated the expression of Bax. The modulation of those two protein expression by polyamine may direct the process of apoptosis in LNCaP cells.

Discussion

The naturally occurring polyamines, including putrescine, spermidine and spermine, are known to play multifunctional roles in cell growth, proliferation and differentiation. Because of their polycationic nature, they bind to nucleic acids and proteins and affect their conformation and biological activity[12]. Recently these polyactions have been involved in the progression of the cell cycle. Polyamine content is altered during the course of cell cycling. Depletion of polyamines would result in cell-growth arrest, mainly at G1 phase. However, in the case where cells are subjected to a culture medium with high levels of polyamines, polyamines exert toxic effects on the cells. In several types of cells, the level of intracellular polyamines seems to be important factors to regulate the cell proliferation and death[21,22]. In the present results, spermidine and spermine at a lower concentration (under 10 μM) stimulated the cell proliferation. The stimulatory effects of spermidine and spermine on cell viability may be, at least in part, resulted from the antioxidant action of polyamines as free radical scavengers[5]. But at a higher concentration (40 μM) they inhibited cell proliferation. These results explain that polyamine homeostasis is critically involved in cellular survival and death, which makes them suitable targets for therapeutic intervention that is specifically directed to cell death pathways [15]. In the present study, putrescine did not give any effect on either the cell viability or intracellular Ca^{2+} level change. In most eukaryotic cells, the total intracellular concentrations of spermidine and spermine are in milimolar range. However, putrescine levels are very low, and in some cells it is difficult to detect. Putrescine, which is the first polyamine produced from ornithine, is used up for the synthesis of spermidine and spermine. Therefore, the possible roles of polyamines are mainly focused on spermidine or spermine. This might be the reason why putrescine at low concentrations or even at very high concentrations

did not show any effect on the cell viability or Ca^{2+} level. In vitro cytotoxicity of spermidine and spermine has been recognized for many years. Cytotoxicity was due to serum amine oxidase, which degrades spermidine and spermine into amino aldehydes, ammonia and hydrogen peroxide, substances that potentially have detrimental effects on cells [9]. Polyamine oxidation catalysed by polyamine oxidase (PAO) and its subsequent product hydrogen peroxide have been implicated in inducing apoptotic cell death. Recently, Schipper et al. suggested that toxicity by high concentrations of polyamine may be independent from the oxidation processes[15]. Increased levels of cytosolic spermine can also directly activate apoptosis that is independent of PAO. Exposure cells to spermine triggered caspase activation and was accompanied by the proteolytic processing of procaspase-3, poly-(ATP-ribose) polymerase (PARP) and an accumulation of cytochrome c in the cytosol. These effects were not blocked by antioxidants or inhibition of PAO but could be abolished by polyamine acetylation.

Ca^{2+} signaling, one of the key regulators of cell survival, is responsible for the regulation of most processes in normal cells. Usually, Ca^{2+} signals may also be important for cell survival mechanism, but the depletion of Ca^{2+} stored in endoplasmic reticulum (ER) and an elevation of cytosolic free Ca^{2+} are involved in the execution of apoptosis. Apoptosis has become a new target for cancer therapy, and abnormal Ca^{2+} signaling is a central feature of tumor cells and a potential target for cancer therapy in that prolonged increase in intracellular Ca^{2+} concentration leads to apoptosis[6]. The elevation of intracellular Ca^{2+} activates a wide variety of Ca^{2+}-sensitive enzymes. These enzymes may generate signaling molecules for the recruitment of mitochondria to the apoptotic cascade or for the activation of the caspase enzymes. Calpains, calcineurin, PKC, and caspases, are among the cytoplasmic targets of Ca^{2+}, which are directly connected to the executioners of apoptosis. Recently, the Bcl-2 family proteins emerge as major regulators of cellular calcium handling[3,11,13,17]. Bcl-2 family proteins play fundamental roles in the integration of apoptotic signals. An overexpression of the antiapoptotic proteins Bcl-2 or Bcl-x_L in many cell types prevents apoptosis through the inactivation of caspases. Conversely, an overexpression of the proapoptotic member Bax triggers the death signals. Bcl-2 family proteins may control membrane permeability through the interaction with channels and membrane transporters. An overexpression of Bcl-2 re-

sults in a reduction of ER-stored Ca^{2+}, whereas a down-regulation of Bcl-2 yields an increase in ER-stored Ca^{2+}. Besides lowering the ER-stored Ca^{2+}, Bcl-2 also blocks Ca^{2+} influx, which contributes to protect cells from intracellular Ca^{2+} increase. Bax inserted itself into the outer membrane of mitochondria and induces the cytochrome c release from mitochondria. Mitochondrial cytochrome c further activates other apoptotic proteins including caspases. Spermine at 10 μM did not give any sign of apoptosis in the morphological assessment. However, at 40 μM spermine, severe nuclear condensation and chromatin fragmentation were found in Hoechst and PI staining. When the intracellular Ca^{2+} level was checked in polyamine-treated cells, all three kinds of polyamines tested induced an increase of intracellular Ca^{2+} level not at lower concentration, but only at high polyamine concentrations. The cytosolic calcium increase was, mainly, due to the influx from the surrounding medium (because no increase in calcium level was found in the experiments with Ca^{2+}-free medium, even at high polyamine concentrations). It is known that endogenous polyamines can modulate several types of ion channels in both excitable and non-excitable cells. In neuron, spermine and spermidine blocked the K^{2+} channel[24]. Spermine could also block Ca^{2+} influx through AMPA/kainite receptor and NMDA receptor. Most intracellular Ca^{2+} move inward through several types of voltage-sensitive channels which are well defined in neuron and muscle cells. However, nonselective cation channel (NSCC) also contributes to the Ca^{2+} influx in many cell systems[4]. NSCC is known to be very sensitive to the surrounding calcium concentration[25]. In the present study, the increase of spermine-induced intracellular Ca^{2+} was not affected at all by nifedipine. However, flufenamic acid completely blocked the spermine-induced Ca^{2+} increase in LNCaP cells. These results indicate that polyamine-induced intracellular Ca^{2+} increase is not due to voltage-sensitive channel, but by NSCC. Polyamines might affect the opening of the NSCC. In hormone-insensitive PC3 human prostate cancer cells, estrogen could increase the Ca^{2+} influx eventhough the exact mechniams are not yet known[7]. That estrogen-induced Ca^{2+} increase might be resulted from the action of NSCC.

An elevation of intracellular Ca^{2+} level may result in an activation of a wide variety of Ca^{2+}-sensitive enzymes. The increase of cytososlic free Ca^{2+} level can directly affect mitochondria to activate apoptotic singnals. These changes

may also generate signaling molecules for recruitment of apoptotic factors or for the activation of the caspase enzymes. The Bcl-2 family proteins are deeply involved in the regulation of apoptosis. Antiapoptotic Bcl-2 protein prevents cytochrome c release, caspase activation, and apoptosis. Conversely, proapoptotic Bax induces apoptosis by disturbing mitochondrial function. Mitochondria are in the center of apoptotic signaling. The release of cytochrome c from mitochondrial intermembrane, membrane potential change, and the opening of mitochondria permeability transition pore are some of the key factors in apoptosis.. In the present results, the expression of antiapoptotic Bcl-2 was not changed at a lower concentration but completely abolished at high concentrations of polyamines. To the contrary, the expression of proapoptotic Bax protein was highly increased in the high polyamine concentration treatment. These findings coincide with results showing the decrease of cell viability and the increase of intracellular Ca^{2+} level at high polyamine concentration.

In summary, polyamines, in particular spermidine and spermine, show dual effects on cell viability; stimulate cell proliferation at low concentrations but inhibit it at high concentrations. At high concentrations, polyamines induce the increase of cytosolic free Ca^{2+} level. The increase in Ca^{2+} level is mainly due to the influx through the nonselective cation channel in the cell membrane. Apoptotic cell death induced by high polyamine concentrations were accompanied by blocking the Bcl-2 expression and by stimulating the Bax expression.

Acknowledgement

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초록 : 전립선암세포에 있어서 폴리아민에 의한 칼슘신호와 세포사멸

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폴리아민은 거의 모든 세포에 있어서 성장과 분화에 필수적인 물질이다. 이들 폴리아민의 기작은 상당히 복잡하고 다양하여 그 정확한 작용기전은 아직 확실하지가 않다. 본 논문에서는 폴리아민이 세포 증식을 유도 하기도 하지만 일정농도 이상에서는 오히려 세포사멸을 초래한다는 결과를 밝히고자 한다. 본 실험에 사용된 인간의 전립선 암세포(LNCaP cells)에 있어서, 폴리아민 가운데 putrescien은 세포 증식에 거의 영향을 미치지 않았으나 spermidine과 spermine의 경우 10 µM 이하에서는 세포 증식을 촉진하였다. 그러나, 20 µM 이상의 농도에서는 농도와 시간의존적으로 세포사멸을 유도 하였다. 폴리아민 처리에 의한 세포사멸의 초기과정인 핵 응축과 염색질 condensation이 Hoechst 와 PI 염색에서 뚜렷이 관찰되었다. 또한, 폴리아민 처리시 anti-apoptotic protein으로 알려진 Bcl-2 protein의 발현은 거의 완전히 억제된 반면, pro-apoptotic protein으로 알려진 Bax의 발현은 현저히 증대되었다. 본 연구의 결과에 따르면, 폴리아민에 의해서 유도되는 세포사멸은 세포 내 칼슘농도 변화에 의한 것으로 사료된다. 전립선 암세포에 있어서 폴리아민 처리시 시간과 농도 의존적으로 세포 내 칼슘농도가 증가되었다. 세포막을 통한 칼슘이동을 억제하는 nifedipine과 flufenamic acid 등의 억제제를 처리한 실험 결과 세포 내 칼슘증가는 세포 내부의 저장소로부터 칼슘의 유출보다는 주로 세포막상에 있는 비선택적 칼슘통로를 통한 외부의 칼슘 유입에 의한 것으로 판단된다.