J. Exp. Biomed. Sci. 12 (2006) 57-63

Cytochrome C Release and Caspase Activation Induced by 3-Deazaadenosisne is Inhibited by Bcl-2

Yong-Joon Lee, Mi-Hyun Choi, Jung-Hee Lee, Ho-Shik Kim† and Jeong-Hwa Lee†

Department of Biochemistry, College of Medicine, The Catholic University of Korea, Seocho-Gu, Seoul 137-701, Korea

Deazaadenosine analogs such as 3-deazaadenosine (DZA), 3-deazaaristeromycin (DZAri) and ara-3-deazaadenine (DZAra-A) were developed as inhibitors of S-adenosylhomocysteine (Ado-Hcy) hydrolase (EC 3.3.1.1). These analogs were reported to induce apoptosis in human and murine leukemic cells. But, the mechanism involved in this apoptosis was not clarified yet. In the present study, we analyze the apoptosis induced by deazaadenosine analogs in human cervival cancer cell line, HeLa and the effect of Bcl-2 on this apoptosis. Whereas neither DZAri nor DZAra-A showed inhibitory effect on HeLa cell growth, DZA induced apoptosis in HeLa cells accompanied by cytochrome c release and activation of various caspases such as caspase-2, -8, -9 and -3. In HeLa-bcl-2 cell line, a stable transfectant of HeLa cell to overexpress Bcl-2, cytochrome c release, activation of all these caspases and the resulted apoptosis by DZA were completely prevented. By *in vitro* assay of cytochrome c release, in addition, DZA induced cytochrome c release from purified mitochondria of HeLa-pcDNA3 cells, but not HeLa-bcl-2 cells, even in the absence of cytosolic fraction. Therefore, it can be suggested that DZA might damage directly mitochondria leading to activate intrinsic pathway of caspase and thus induce apoptosis. DZA-induced apoptosis in HeLa cells may be in a bcl-2-inhibitable manner and irrelative of Ado-Hcy hydrolase.

Key Words: Apoptosis, Caspase, Cytochrome C, Bcl-2, S-adenosylhomocysteine hydrolase

INTRODUCTION

Deazaadenosine analogs such as 3-deazaadenosine (DZA), 3-deazaaristeromycin (DZAri) and ara-3-deazaadenine (DZAra-A) were originally developed as inhibitors of S-adenosylhomocysteine (Ado-Hcy) hydrolase (EC 3.3.1.1) (Chiang et al., 1977). Among these analogs, DZA, the weakest inhibitor binds to Ado-Hcy hydrolase resulting in accumulation of Ado-Hcy and S-adenosylmethionine (Ado-Met), and also serves as a substrate for that enzyme resulting in huge accumulation of 3-deazaadenosylhomocysteine (DZA-Hcy) in cultured cells and the liver (Chiang et al., 1977; Aksamit et al., 1982). Besides its function as an inhibitor of Ado-Hcy hydrolase, DZA exerts a number of interesting biological functions such as anti-human immunodeficiency virus (HIV) activity (Mayers et al.,1995), immunosuppressive and anti-inflammatory effects (Krenitsky et al., 1986), inhibition of lymphocyte-mediated cytolysis (Zimmerman et al., 1978), inhibition of cytokine expression including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (Jeong et al., 1996), inhibition of cell adhesion molecule expression (Shankar et al.,1992) and inhibition of nuclear factor-κB (NF-κB) transcriptional activity (Jeong et al., 1999). In addition, DZA has been reported to induce apoptosis in human and murine leukemic cell lines (Endresen et al., 1993; Kim et al., 1997).

Although it was reported that DZA induces apoptosis in human leukemic cells such as U937 and HL-60 by activating caspases (Kim et al., 2000), apoptosis-inducing activity of DZA was not demonstrated in human solid cancer cell lines and the mechanism based on molecular events in the DZA-induced apoptosis has not been fully elucidated.

Thus, to address these issues, we have investigated the

*Received: March 8, 2006
Accepted after revision: April 4, 2006
†Corresponding author: Ho-Shik Kim and Jeong-Hwa Lee, Department of Biochemistry, College of Medicine, The Catholic University of Korea, Seocho-Ku, Seoul 137-701, Korea.
Tel: 02-590-1180, Fax: 02-596-4435
e-mail: hoshik@catholic.ac.kr

apoptotic mechanism of DZA in human cervical cancer cell line (HeLa) and analyzed the effect of bcl-2 by comparing the apoptosis in HeLa-pcDNA3, a stable transfectant of empty pcDNA3 vector with that in HeLa-bcl-2, a stable transfectant expressing exogenous bcl-2, on the DZA-induced apoptosis in the present study.

MATERIALS AND METHODS

1. Chemicals

Deazaadenosine analogs were kind gifts of Dr. Chiang of the Walter Reed Army Institute of Research, Silver Spring, MD. Protease inhibitor cocktail was from Roche Molecular Biochemicals (Mannheim, Germany). 3-(4,5-Dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) was from Sigma (St. Louis, MO). All other reagents were of ACS or molecular biology grade, and from Sigma, otherwise specified.

2. Cell culture and generation of stable cell lines

HeLa cells purchased from American Type Tissue Culture (ATCC, Rockville, MD) were maintained in RPMI1640 media supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT) and penicillin/streptomycin (100 units/mL) in a humidified atmosphere of 95% air and 5% CO_2. PcDNA3 and pcDNA3-bcl-2 was transfected into HeLa cells, respectively, by lipofectamine. Stable transfectants were selected in the presence of G418 (500 μg/ml).

3. Assay of antiproliferative activity

To observe the effect of DZA on the growth of HeLa cells, cell viability was determined by MTT test. Cells were seeded at a density of 1×10^4/well in 96-well culture plates. After overnight incubation, cells were treated with indicated concentrations of DZA. After 6 hours, 10 μL of MTT reagent was added to each well and three hours later, MTT crystals were dissolved with isopropanol containing HCl (0.04 N) and absorbance at 570 nm was measured.

4. Evaluation of apoptosis

To examine the integrity of genomic DNA, total cellular DNA was extracted from 7.5×10^5 cells treated with DZA. Briefly, cells were lysed in lysis buffer (500 mM Tris-Cl, pH 9.0, 2 mM EDTA, 10 mM NaCl) containing 1% (w/v) sodium dodecyl sulfate and treated with proteinase K (100 μg/mL) for 24 hours. DNA was extracted phenol/chloroform/isoamylalcohol (25:24:1, v/v/v) and absolute ethanol. Equal amounts of extracted DNA were electrophoresed in 1.5% agarose gel and examined under UV light after stained with ethidium bromide.

5. Antibodies

Mouse anti-PARP antibody was obtained from Serotec Inc. (Raleigh, NC). Rabbit anti-caspase-3, -2, -8 and -9 antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Secondary antibodies against rabbit IgG and mouse IgG were from Sigma.

6. Western blot analysis

After treatments, cells were harvested and washed with PBS. And then cells were lysed with RIPA buffer [1% TritonX-100, 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1 mM EDTA] containing protease inhibitor cocktail. Protein concentrations were measured by Bicinchoninic Acid (BCA) method (Pierce Biotechnology, Inc., Rockford, IL). Fifty micrograms of protein were resolved on 10% SDS-polyacrylamide gel electrophoresis and electro-transferred onto nitrocellulose membranes (Schleicher and Shuell, Dassel, Germany). Membranes were soaked in 5% non-fat dry milk in tris-buffered saline (TBS; 10 mM Tris-HCl, pH 7.5, 150 mM NaCl) for 1 hour and incubated with primary antibodies at 4℃ overnight. After washing in TTBS (TBS containing 0.05% of Tween 20) four times, membranes were subsequently incubated with horseradish peroxidase-conjugated secondary antibodies (either goat anti-mouse or goat anti-rabbit) at room temperature. One hour later membranes were washed in TTBS and proteins were detected by enhanced chemiluminescence (ECL, Amersham, Buckinghamshire, UK) according to the manufacturer's instructions.

7. Subcellular fractionation

Subcellular fractionation was performed according to the method of Akao et al. (1994). Briefly, harvested cells were washed twice with ice-cold PBS and then resuspended in hypotonic buffer (10 mM HEPES, 10 mM $MgCl_2$, 42 mM KCl). Cells were passed through a 30-gauge syringe and centrifuged at 1,000 rpm for 5 min to remove unlysed cells and nuclei. The supernatant was further centrifuged at 15,000 rpm for 15 min at 4℃. The resulted pellet is heavy

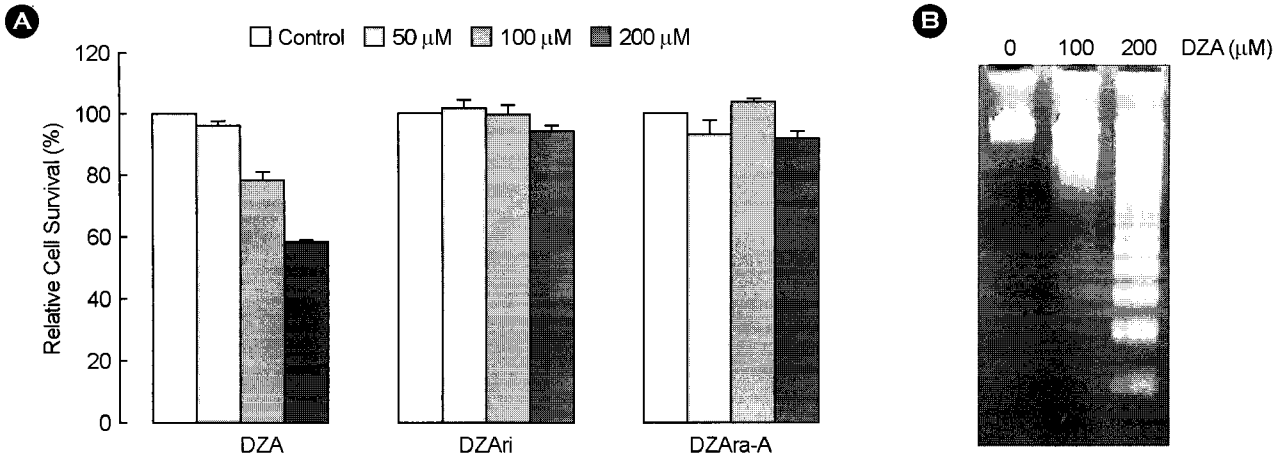

Fig. 1. Induction of apoptosis by DZA in HeLa cells. **(A)** HeLa cells were treated with the indicated concentrations of DZA, DZAri and DZAra-A. After 6 hours, MTT assay was performed as described in Materials and Methods and expressed as relative cell survival which means the percent of cell viability of the treated cells to that of the cells without treatment. The data were expressed as mean ± S.D. of three independent experiments performed in triplicate. **(B)** Total cellular DNA of HeLa cells treated with indicated concentrations of DZA was extracted as described in Materials and Methods. Extracted DNA were separated in 1.5% agarose gel.

membrane fraction used as the mitochondrial fraction. The supernatant was ultracentrifuged at 100,000 g for 90 min. The pellet is light membrane fraction and the supernatant is cytosolic fraction.

RESULTS

1. Induction of apoptosis by DZA in HeLa Cells

We tested effect of Ado-Hcy hydrolase inhibitors such as DZA, DZAri and DZAra-A on the growth of HeLa cells by MTT assay. As shown in Fig. 1, DZA inhibited the growth of HeLa cells dose dependently while neither DZAri nor DZAra-A exerted growth inhibitory activity. On agarose gel electrophoresis, total cellular DNA of cells treated with DZA was shown to be fragmented suggesting that DZA inhibit the growth of HeLa cells by inducing apoptosis.

2. Inhibition of DZA-induced apoptosis by Bcl-2

To confirm the cell death mode of DZA-induced HeLa cell death and further characterize the mechanisms involved in this model, we analyzed the effect of Bcl-2, a representative inhibitor of apoptosis. HeLa cells overexpressing Bcl-2 were resistant to growth inhibition by DZA and ladder DNA formation by DZA was almost completely blocked in HeLa-bcl-2 cells (Fig. 2). These findings suggest that DZA-induced cell death is apoptosis which could be inhibited by bcl-2.

3. Activation of caspase cascade in DZA-induced apoptosis

Bcl-2 is known to inhibit apoptosis by blocking cytochrome c translocation from mitochondria to cytosol leading to activation of caspase cascade (Reviewed in Antonsson, 2005). So, it may be possible to identify caspase cascade pathway induced by DZA by comparing caspase activation pathway in HeLa cells to that in HeLa-bcl-2 cells. To address this assumption, we checked the processing of procaspases during this apoptosis induction. As shown in Fig. 3, initiator caspases such as caspase-2, caspase-8 and caspase-9 and an effector caspase, caspase-3 were activated and PARP a substrate of caspase-3 was cleaved in HeLa-pcDNA3 cells by DZA treatment implying that DZA might activate initiator caspases such as caspase-2 and -8 which signal cytochrome c release and then activation of caspase-9 which in turn finally activate caspase-3. However, in HeLa-bcl-2 cells whose cytochrome c release by activated initiator caspsases can not be taken place due to bcl-2 overexpression, neither caspases upstream of mitochondrial event such as caspase-2 and -8 nor caspases downstream of mitochondrial event such as caspase-9 and -3 were activated by DZA. Therefore, in this DZA-induced apoptosis of HeLa cells, cytochrome c translocation seems to be located upstream of caspase-2 and caspase-8 and play a critical role in initiation of apoptosis. Notably, Bip whose expression is increased by endoplasmic reticulum (ER) damage was not altered implying

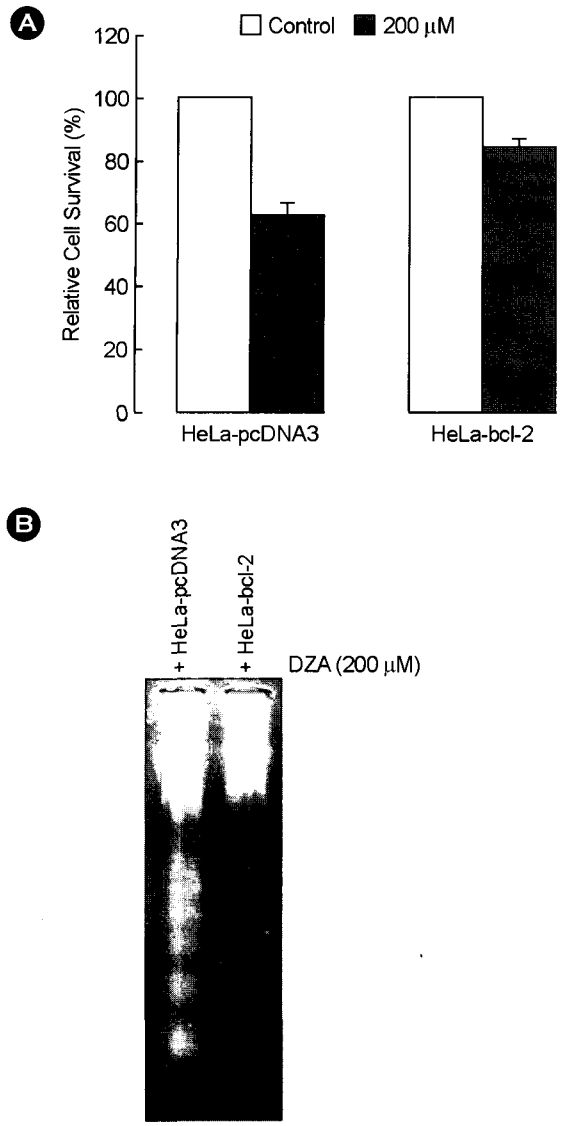

Fig. 2. Inhibition of DZA-induced apoptosis by Bcl-2. **(A)** HeLa-pcDNA3 and HeLa-bcl-2 cells were treated with 200 μM of DZA. After 6 hours, MTT assay was performed as described in Materials and Methods and expressed as relative cell survival which means the percent of cell viability of the treated cells to that of the cells without treatment. The data were expressed as mean ± S.D. of three independent experiments performed in triplicate. **(B)** Total cellular DNA of HeLa-pcDNA3 and HeLa-bcl-2 cells treated with 200 μM of DZA for 6 hours were extracted and eletrophoresed in 1.5% agarose gel as described in Materials and Methods.

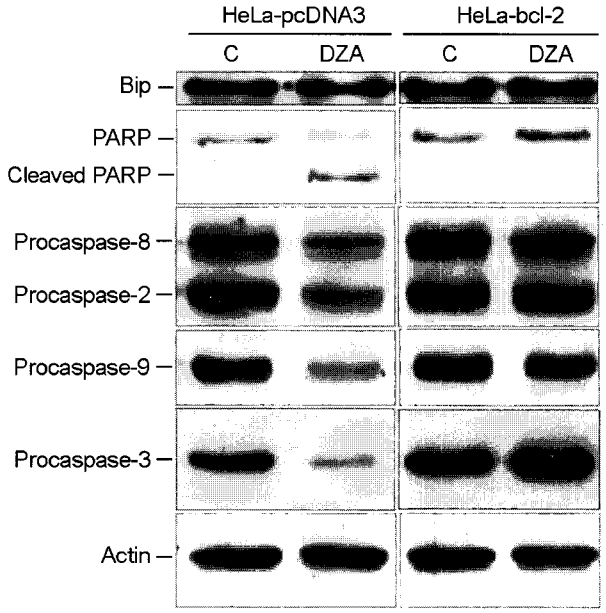

Fig. 3. Cleavage of PARP and activation of caspases by DZA. HeLa-pcDNA3 and HeLa-bcl-2 cells were incubated with or without 200 μM of DZA. After 6 hours, total cell lysates were prepared and subjected to 10% SDS-PAGE and then immunoblot analysis was performed as described in Material and Methods. Actin was used for equal protein amount of loaded samples.

that this apoptosis may be purely linked with mitochondria-associated caspase cascade.

4. Induction of cytochrome c release by DZA

To understand the mechanism of cytochrome c release by DZA, we checked expression distribution of cytochrome c and Bax in DZA-treated cells. As expected, cytochrome c was translocated from mitochondria to cytosol by DZA treatment only in HeLa-pcDNA3 cells, but not in HeLa-bcl-2 cells. Bax which potentiates cytochrome c release, was translocated from cytosol to mitochondria by DZA treatment in HeLa-pcDNA3 cells but not in HeLa-bcl-2 cells suggesting Bax translocation may be precedent to mitochondrial cytochrome c release and Bcl-2 may inhibit Bax translocation resulting in apoptosis inhibition. But, it is also thinkable that Bax translocation is below the mitochondrial cytochrome c release and caspase activation, and the inhibition of Bax translocation is a resultant phenomenon of apoptosis inhibition by bcl-2 overexpression. To clarify these suggestions and confirm the requirement of cytosolic component including Bax protein for cytochrome c release by DZA, *in vitro* cytochrome c release assay was performed. As shown in Fig. 4B, DZA induced cytochrome c release from purified mitochondria of HeLa-pcDNA3 cells, but not HeLa-bcl-2 cells, even in the absence of cytosolic component as well as in the presence of cytosol (data not shown), implying that the cytochrome c release by DZA might be due to a direct interaction of DZA with mitochondria and Bax translocation to mitochondria may not be the primary event to induce apoptosis by DZA.

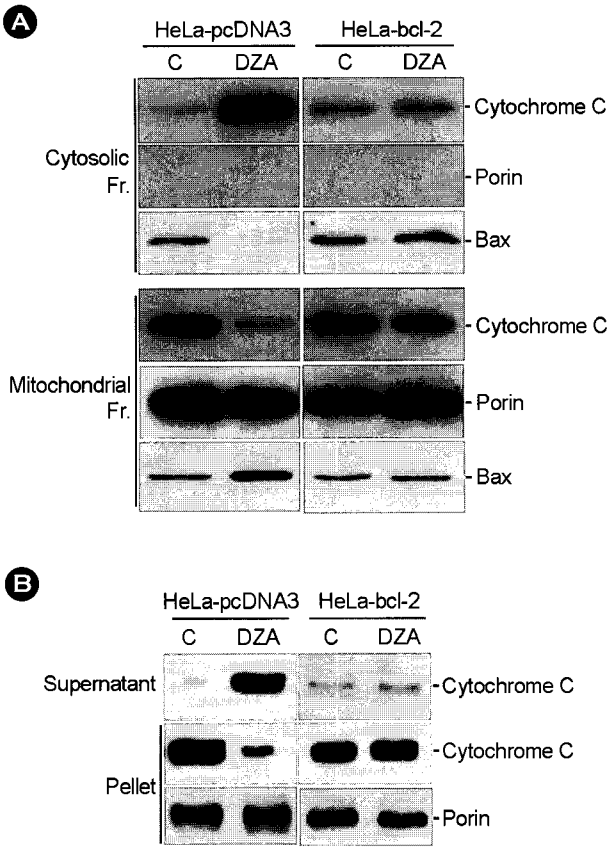

Fig. 4. Release of cytochrome c induced by DZA. **(A)** Subcellular fractions of HeLa-pcDNA3 and HeLa-bcl-2 cells treated with or without 200 μM of DZA for 6 hours were prepared and subjected to immunoblot analysis as described in Material and Methods. **(B)** *In vitro* assay of cytochrome c release. Purified mitochondria of HeLa-pcDNA3 and HeLa-bcl-2 cells were incubated in the resusepnsion buffer containing no or 200 μM of DZA for 6 hours. Mitochondrial suspension was then centrifuged to separate pellet and supernatant fractions, which were subjected to immunoblot analysis as described in Material and Methods. Porin was used for adequate fractionation of cytosol and mitochondria, and equal loading of proteins.

DISCUSSION

Deazaadenosine analogues such as DZA, DZAri and DZAra-A were developed as inhibitors of S-adenosylhomocysteine (Ado-Hcy) hydrolase (EC 3.3.1.1). But, many biological functions of these inhibitors, especially DZA were reported to be unrelated with S-Ado-Hcy hydrolase. In apoptosis model presented here, only DZA which is less effective than DZAri and DZAra-A on inhibition of that enzyme, induced apoptosis. So, DZA-induced apoptosis seems not to be related with inhibition of S-Ado-Hcy hydrolase.

It has been reported that DZA can induce apoptosis in human and murine leukemic cells of which mechanism was not clarified yet. Although Kim et al. (2000) suggested that DZA may activate caspase-3-like enzyme to induce apoptosis, the order of caspase activation and the primary event of DZA-induced apoptosis still remain obscure.

In HeLa cells treated by DZA, apoptosis, cytochrome c release and activation of caspases including caspase-8, -2, -9 and -3 were observed. And Bax which can form pores in outer mitochondrial membranes through which cytochrome c is released were translocated to mitochondria by DZA treatment. But, in HeLa-bcl-2 cells, all the above phenomena were almost completely prevented and by *in vitro* assay, cytochrome c was released from purified mitochondria even in the absence of cytosolic component. Based on these findings we may recapitulate intracellular events taken place during DZA-induced HeLa cell apoptosis as follows. Intracellular DZA may target to mitochondria, where stimulates the release of cytochrome c. Released cytochrome c may in turn induce activation of caspase cascade, finally resulting in apoptosis.

Although the release mechanism of cytochrome c which is normally sequestered in mitochondrial intermembrane space to cytosol still remains as one of the hotly debating issues in apoptosis field, it is well demonstrated that cytochrome c release can be induced via permeability transition (PT) pores associated with mitochondrial outer membrane permeabilization (MOMP) (Reviewed in Green and Kroemer, 2004). PT pores are composed of the adenine nucleotide transporter (ANT) in the inner mitochondrial membrane, the voltage-dependent anion channel (VADC) in the outer mitochondrial membrane and other putative proteins. Opening of PT pores by apoptotic triggers induce mitochondrial swelling and then rupture of outer mitochondrial membrane leading to release of components in intermembrane space including cytochrome c. Bcl-2 can perturb this series of events to block the release of cytochrome c, of which mechanism in detail is under investigation. So, it can be expected that DZA might play a role in MOMP to induce Bcl-2-dependent apoptosis. Moreover, it has been reported that deoxyadenosine analogs directly disrupted the integrity of mitochondria, probably by binding of deoxyadenosine analogs with the ANT, before inducing apoptosis in leukemic cells (Genini et al., 2000). Then, it can be postulated that DZA might bind to the ANT or other mitochondrial proteins and thus stimulate the release of cytochrome c, but

more intensive experiments with fine molecular biological techniques should be performed to understand molecular mechanisms involved in the release of cytochrome c.

It is generally accepted that caspase-8 and caspase-2 function as initiator caspases, being upstream of mitochondrial event during apoptosis. Caspase-8 relays death signals generated by Fas receptor-ligand interaction to downstream caspase-3, directly cleaving procaspase-3 or to mitochondria via truncated Bid (Kruidering and Evan, 2000). In addition, it has been shown that caspase-8 itself can cleave many other caspases (Srinivasula et al., 1996), implying that caspase-8 acts as an initiator caspase and is upstream of cytochrome c release. In the present study, however, caspase-8 activation by DZA in HeLa cells was completely prevented by Bcl-2, strongly suggesting that caspase-8 activation may be located downstream of cytochrome c release. The mechanism involved in the activation of caspase-8 was not clarified in this study but this finding may be in the same vein with other reports that caspase-8 is a downstream effector of caspase-3 or -9 (Slee et al., 1999; Wieder et al., 2001). Caspase-2 is activated in early response to many apoptotic stimuli such as DNA-damaging drugs and TNF-α. Recent reports suggested that caspase-2 is responsible for permeabilization of mitochondria to amplify cytochrome c release, especially in the apoptosis by DNA-damaging agents such as etoposide (Robertson et al., 2002). But, its activation in DZA-induced apoptosis was completely prevented by inhibition of cytochrome c release due to bcl-2 overexpression ruling out the preceding role of caspase-2 on cytochrome c release and apoptosis. In addition, when we observed the effect of zVAD-Fmk, a pan-caspase inhibitor, on the cytochrome c release by DZA, to determine the caspase activation involved in cytochrome c release, cytochrome c was tranlocated from mitochondria even in the presence of zVAD-Fmk (data not shown). Considering that DNA-damage response can be initiated by apoptotic DNA fragmentation, it can be suspected that caspase-2 may be activated by fragmented DNA downstream of mitochondrial event, although the role of caspase-2 was not clarified in this DZA-induced apoptosis.

Taken together, DZA may induce apoptosis in HeLa cells in a somewhat peculiar way in terms of order of caspase activation and cytochrome c release. To confer physiological significances of this pathway, molecular biological experiments to identify the effect of specific knock-down of each caspase on this apoptosis should be performed. This investigation in the future would be able to contribute to under stand the mechanism of apoptosis in general as well as apoptosis-induced by DZA, which can lead to determine the applicability of DZA for treatment of human diseases like cancer and to develop other drugs based on this mechanism.

Acknowledgements

The authors wish to acknowledge the financial support of the Catholic Medical Center Research Foundation made in the program year of 2005.

REFERENCES

Antonsson B. Mitochondria and the Bcl-2 family proteins in apoptosis signaling pathways. Mol Cell Biochem. 2004. 256: 141-155.

Akao Y, Otsuki Y, Kataoka S, Ito Y, Tsujimoto Y. Multiple subcellular localization of bcl-2: detection in nuclear outer membrane, endoplasmic reticulum membrane, and mitochondrial membranes. Cancer Res. 1994. 54: 2468-2471.

Aksamit RR, Backlund PS, Cantoni GL. Inhibition of chemotaxis by s-3-deazaadenosylhomocysteine in a mouse macrophage cell line. J Biol Chem. 1982. 257: 621-625.

Chiang PK, Richards HH, Cantoni GL. S-Adenosyl-L-homocysteine hydrolase; analogues of S-adenosyl-L-homocysteine as potential inhibitors. Mol Pharmacol. 1977. 13: 939-947.

Endresen PC, Eide TJ, Aarbakke J. Cell death initiated by 3-deazaadenosine in HL-60 cells is apoptosis and is partially inhibited by homocysteine. Biochem Pharmacol. 1993. 46: 1893-1901.

Genini D, Adachi S, Chao Q, Rose DW, Carrera CJ, Cottam HB, Carson DA, Leoni LM. Deoxyadenosine analogs induce programmed cell death in chronic lymphocytic leukemia cells by damaging the DNA and by directly affecting the mitochondria. Blood 2000. 96: 3537-3542.

Green DR, Kroemer G. The Pathophysiology of Mitochondrial Cell Death. Science 2004. 305: 626-629.

Jeong SY, Ahn SG, Lee JH, Kim HS, Kim JW, Rhim H, Jeong SW, Kim IK. 3-Deazaadenosine, a s-adenosylhomocysteine hydrolase inhibitor, has dual effects on NF-κB regulation. J Biol Chem. 1999. 274: 18981-18988.

Jeong SY, Lee JH, Kim HS, Hong SH, Cheong CH, Kim IK. 3-Deazaadenosine analogues inhibit the production of tumor

necrosis factor-alpha in RAW264.7 cells stimulated with lipopolysaccharide. Immunology 1996. 89: 558-562.

Kim HS, Jeong SY, Lee JH, Kim BE, Kim JW, Jeong SW, Kim IK. Induction of apoptosis in human leukemia cells by 3-deazaadenosine is mediated by caspase-3-like activity. Exp Mol Med. 2000. 32: 197-203.

Kim IK, Li CCH, Young HA, Lee JH, Kim HS, Pardhasaradhi K, Garcia GE, Chiang PK. Apoptosis of L1210 leukemia cells induced by 3-deazaadenosine analogs: differential expression of c-myc, NF-κB and molecular events. J Biomed Sci. 1997. 4: 83-90.

Krenitsky TA, Rideout JL, Chao EY, Koszalka GW, Gurney F, Crouch RC, Cohn NK, Wolberg G, Vinegar R. Imidazo [4,5-c]pyridines (3-deazapurines) and their nucleosides as immunosuppressive and anti-inflammatory agents. J Med Chem. 1986. 29: 138-143.

Kruidering M, Evan GI. Caspase-8 in apoptosis: the beginning of "the end"?. IUBMB Life 2000. 50: 85-90.

Mayers DL, Mikovits JA, Joshi B, Hewlett IK, Estrada JS, Wolfe AD, Garcia, GE, Doctor BP, Burke DS, Gordon RK, Lane JR, Chiang PK. Antihuman immunodeficiency virus 1 (HIV-1) activities of 3-deazaadenosine analogs: increased potency against 3'-azido-3'-deoxythymidine-resistant HIV-1 strains. Proc Natl Acad Sci USA. 1995. 92: 215-219.

Medzihradsky JL. Regulatory role for the immune complex in modulation of phagocytosis by 3-deazaadenosine. J Immunol. 1984. 133: 946-949.

Robertson JD, Enoksson M, Suomela M, Zhivotovsky B, Orrenius S. Caspase-2 acts upstream of mitochondria to promote cytochrome c release during etoposide-induced apoptosis. J Biol Chem. 2002. 277: 29803-29809.

Shankar R, de la Motte CA, DiCorleto PE. 3-Deazaadenosine inhibits thrombin-stimulated platelet-derived growth factor production and endothelial-leukocyte adhesion molecule-1-mediated monocytic cell adhesion in human aortic endothelial cells. J Biol Chem. 1992. 267: 9376-9382.

Slee EA, Herte MT, Kluck RM, Wolf BB, Casiano CA, Newmwyer DD, Wang HG, Reed JC, Nicholson DW, Alnemri ES Green DR, Martin SJ. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. J Cell Biol. 1999. 144: 281-292.

Srinivasula MS, Manzoor A, Teresa FA, Gerald L, Emad SA. Molecular ordering of the Fas-apoptotic pathway: the Fas/APO-1 protease Mch5 is a crmA-inhibitable protease that activates multiple ced-3/ICE-like cysteine proteases. Proc Natl Acad Sci USA. 1996. 93: 14486-14491.

Wieder T, Essmann F, Prokop A, Schmeiz K, Schulze-Osthoff K, Beyaert R, Dörken B, Daniel PT. Activation of caspase-8 in drug-induced apoptosis of B-lymphoid cells is independent of CD95/Fas receptor-ligand interaction and occurs downstream of caspase-3. Blood 2001. 97: 1378-1387.

Zimmerman TP, Wolberg G, Duncan GS. Inhibition of lymphocyte-mediated cytolysis by 3-deazaadenosine. Proc Natl Acad Sci USA. 1978. 75: 6220-6224.