CDST, a Derivative of Tetrahydroisoquinoline, Induced Apoptosis in HL-60 Cells through Activation of Caspase-8, Bid Cleavage and Cytochrome c Release

Sung Min Ju, Kun Jung Kim, Jong Gil Lee, Chai Ho Lee[1], Dong Min Han[1], Young Gab Yun[2], Gi Yun Hong[3], Won Gun An[4], Byung Hun Jeon*

Department of Pathology, College of Oriental Medicine, Wonkwang University,
1: Division of Natural Science and Technology, College of Natural Sciences,
2: Department of Prescription, College of Oriental Medicine, Wonkwang University,
3: Department of Obstetric and Gynecology, College of Medicine, Wonkwang University,
4: Department of Oral Pathology, College of Dentistry, Pusan National Universsity

The tetrahydroisoquinolines included potent cytotoxic agents that showed antitumor activity,antimicrobial activity, and other biological properties. We studied the effect of CDST, 1-Chloromethyl-6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-sulfonic acid amide, a newly synthesized anti-cancer agent. The cytotoxic activity of CDST in HL-60 cells was increased in a dose-dependent manner. CDST, tetrahydroisoquinolines derivative, was cytotoxic to HL-60 cells, with IC50 of 80㎍/㎖. Treatment of CDST to HL-60 cells showed the fragmentation of DNA in a dose- and time dependent manner, suggesting that thesecells underwent apoptosis. Treatment of HL-60 cells with CDST was induced in a dose- and time-dependent activation of caspase-3, caspase-8 and proteolytic cleavage of poly(ADP-ribose) polymerase. In caspase activity assay, caspase-3 and -8 was activated after 12 h and 6 h posttreatment, respectively. CDST also caused the release of cytochrome c from mitochondria into the cytosol. CDST-induced cytochrome c release was mediated by caspase-8-dependent cleavage of Bid and Bax translocation. These results suggest that caspase-8 induced Bid cleavage and Bax translocation, caused mitochondrial cytochrome c release, and induce caspase-3 activationduring CDST-induced apoptosis in HL-60 cells.

Key words : CDST, Tetrahydroisoquinoline, Apoptosis, HL-60

Introduction

Tetrahydroisoquinoline (TIQ) alkaloids, based isoquinoline skeleton, have been various biological activity according to the structure. TIQ alkaloids get rise to much interest because biological activity such as antitumor, anti-HIV, antimalarial, renal vasoconstriction, antihypertensive, Parkinson's disease inhibitor, anticonvulsant and PNMT inhibitor[1-15]. Isoquinoline was deriviatived at quinoline by Hoggewerff and Van Drop in 1885. They have been prepared via the Bischer-Napieralski reaction, Pictet-Spengler reaction, and Pomeranz-Fritsch reaction[16].

The compounds included TIQ skeleton play an important role to precursor in alkaloid synthesis, and it is able to synthesize diverse hetero cyclic compounds and TIQ derivatives. The alkaloid mean biological activity matter with nitrogen gained from basic nitrogen compound of plant and animal or microorganism, and TIQ alkaloid with various biological activity are become the object of interest in various sphere such as medical supplies. 1-Chloromethyl-6, 7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-sulfonic acid amide, CDST used in the study synthesized included aminosulfonyl group in nitrogen of tetrahydropyridine ring using intramolecular α-Sulfamidoalkylation reaction. Synthesized CDST have together with isoquinoline skeleton and ring type sulfamide carring biological activity. The derivative of TIQ, CDST, has been developed as a new anti-cancer agent ,and CDST was cell-specifically cytotoxic to promyelocytic leukemia (HL-60) cells. However, the mechanism of action of CDST remains unknown.

Some anti-cancer agents cause cell deaty by apoptosis[17-21].

* To whom correspondence should be addressed at : Byung Hun Jeon, Department of Pathology, College of Oriental Medicine, Wonkwang University, Iksan 570-749, Korea
· E-mail : omdjbh@wonkwang.ac.kr, · Tel : 063-850-6843
· Received : 2005/03/23 · Revised : 2005/04/25 · Accepted : 2005/05/25

Apoptosis is known as programmed cell death and follows the activation of signal transduction, and a selective physiological process that plays an important role in the balance between cell proliferation and cell death[22-24]. Apoptosis is an important phenomenon in cancer chemotherapy, because anti-cancer agent exert their antitumor effect against cancer cells by inducing apoptosis[25].

Typically two different pathways leading to apoptosis have been identified, namely receptor-mediated and chemical-induced apoptosis[26-28]. Most cytokines (usually members of the TNF superfamily) induceapoptosis by interaction of the ligand with its death receptor, which sequentially recruits TNF receptor-associated death domain; Fas-associated death domain (FADD/MORT1); FADD-like interleukin-1 converting enzyme (FLICE) (also called caspase-8), and caspase-3; the last then cleaves various substrates leading to apoptosis, In contrast, chemical (most chemotherapeutic agents)-induced apoptosis involves cleavage of Bid by caspase-8, which cause the release of cytochrome c from the mitochondria. Once released, cytochrome c bind to Apaf-1 and activates caspase-9 in the presence of dATP[29-31]. The activated caspase-9 leads to the activation of downstream effector caspase, such as caspase-3, which cleaves a number of cellular proteins to execute cell death. Generally, in chemical-induced apoptosis, Bid, activated by caspase-8, is translocated to the mitochondria and induces the release of cytochrome c, but in another chemical-induced apoptosis, cytochrome crelease is caspase-independent and is not mediated by cleavage of Bid[27,32,33]. It has recently been shown that Bax can directly induce cytochrome c rlease from mitochondria without requirement for caspases[34]. The chemical-induced apoptotic pathway involving mitochondira has also been shown to be negatively regulated by antiproapoptotic protein such as Bcl-2 through suppression of cytochrome c release.

Bax, a proapoptotic members of the Bcl-2 family, has been known to cause cytochrome c release from mitochondria and caspase activation in cell-free extracts and in cells treated with apoptosis-inducing agents[34-37]. In addition, Bax translocates from its predominantly cytoplasmic location to the mitochondria upon apoptosis induction[35,38]. In contrast, antiapoptotic Bcl-2 can block cytochrome c release in cells undergoing apoptotis[36,39]. The antiapoptotic Bcl-2 family reside on the outer mitochondrial membrane and can inhibit apoptosis by many mechanisms such as homo- or heterodimerization with other family members, maintenance of normal mitochondrial membrane resulting in the prevention of cytochrome c release and subsequent caspase activation.

In this study, we investigated the cellular mechanisms of cell death in HL-60 cells induced by CDST. We show that CDST induces apoptotic cell death in HL-60 cells by caspase activation following cytochrome c release. It demonstrated that CDST-induced apoptotic cell death activated caspase-3 through caspase-8, and released cytochrome c from mitochondria via Bid cleavage and Bax translocation in HL-60 cells.

Materials and Methods

1. Chemicals and cell culture

1-Chloromethyl-6,7-dimethoxy-3,4-dihydro-1H-isoquinoline -2-sulfonic acid amide (CDST) was prepared by the use of iminium ions as intermediates through α-sulfamidoalkylation. (Fig. 1.) CDST was dissolved in DMSO at a concentration 40 ㎎/㎖ and stored at -20℃ and diluted in cell culture medium before use. The human lymphoma (U-937) and human leukemia (Jurkat, Molt-4, HL-60) cell lines used in this study was obtained from Korean Cell Line Bank (KCLB, Seoul, Korea) and routinely cultured under a humidified atmosphere of 5% CO_2-95% air at 37℃ in RPMI 1640 medium (GIBCO BRL) supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 200 ㎍/㎖ penicillin, and 100 ㎍/㎖ streptomycin.

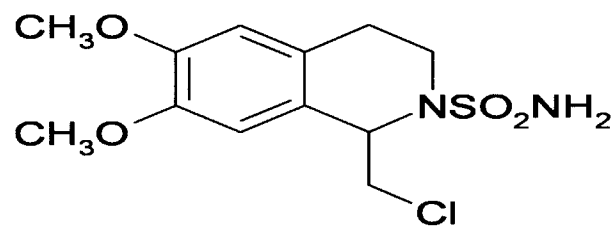

Fig. 1. Structure of CDST.

2. Treatment of cells

CDST (dissolved in DMSO, 40 ㎎/㎖) was used for the treatment of cells. For dose-dependent studies, the cells (2×10^5 cells/㎖) were treated with CDST at 40, 80, 120, and 160 ㎍/㎖ for 24 h in complete cell medium, whereas for time-dependent studies, the cells were treated with a 80 ㎍/㎖ dose of CDST for 6, 12, 18, and 24 h. The control cells were incubated with the highest amount of DMSO used for 24 h for the dose-dependent study. For time-dependent study, in addition to the 0 h control.

3. Cell viability

Exponentially growing cells were seeded into a 24 well plate at 1×10^5 cells/well in duplicate. The cells were treated with increasing concentrations of CDST for 24 hr. After the indicated time periods, 100 ㎕ of 5 ㎎/㎖ MTT (Sigma

Chemicals Co.) was added to each wells and incubated for 4h. Water-insoluble MTT-formazan crystalswere solubilized by adding equal volume of solubilization solution (10% SDS/0.01 N HCl) and incubating the plate overnight in humidified atmosphere of 5% CO_2 at 37℃. The amount of formazan was determined by ELISA reader (SpectraMAX, Molecular Devices) at 570 ㎚.

4. DNA fragmentation analysis

DNA was purified with the Wizard®Genomic DNA Purification Kit (Promega). Briefly, HL-60 cells (2×10^5 cells/㎖) in 10-Φdishes were treated with various cocentration or time of CDST. Treated cells were washed twice with ice-cold PBS and DNA was purified according to some modified manufacturer's protocol and rehydrated in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) by incubating overnight at 4℃. DNA was analyzed after separation by gel electrophoresis (1.2% agarose gel contained 0.5 ㎍/㎖ ethidium bromide at 100 V for 2 h). DNA bands were visualized under UV transilluminator.

5. DAPI staining

The HL-60 cells (2×10^5 cells/㎖) were cultured in 6-well plate in RPMI 1640 medium and HL-60 cells were incubated with various concentrations of CDST for 24 h or with 80 ㎍/㎖ CDST at various time interval. Cells were attached to slides by cytospin, washed with DAPI-methanol (1 ㎍/㎖, Roche), and then stained with DAPI-methanol for 15 min at 37℃. After, stained cells were washed once with methanol and observed under a fluorescence microscope.Apoptotic cells were identified by features characteristic of apoptosis (e.g. nuclear condensation, formation of membrane blebs and apoptotic bodies)

6. Subcellular fractionation

Subcellular fractionation was carried out as described by previous report[40]. After CDST treatment for 24 hr, cells (4×10^6) were harvested, washed once in ice-cold phosphate-buffered saline (PBS), and gently lysed for 30 sec in 80 ㎕of ice-cold lysis buffer (250 mM sucrose, 1 mM EDTA, 0.05% digitonin, 25 mM Tris-HCl, pH 6.8, 1 mM DTT) with freshly added 1% protease inhibitor cocktail (Sigma). Lysates were centrifuged at 11,000 rpm at 4℃ at 4 min. to obtain the supernantants (cytosolic fraction free of mitochondria) and the pellet (the fraction that contains mitochondria). Supernatants and pellets were electrophoresed on a 15% polyacrylamide gell and then analyzed by western blot using mouse monoclonal anti-cytochrome c (BD Pharmingen) and rabbit polyclonal anti-Bax (Santa Cruz) antibodies and SuperSignal West Pico Chemiluminescent (PIERCE).

7. Western blot analysis

Cells were washed with ice-cold PBS and gently resuspend in ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100) with freshly added 1% protease inhibitor cocktail (Sigma Chemicals Co.) and incubated on ice for 30 min. Cell lysates were centrifuged at 14,000 rpm for 10 min at 4℃, and the protein concentration was determined using a Bradford assay. After SDS-PAGE, proteins were transferred to nitorcellulose (Schleicher&Schuell) for 3 hr at 40V. Blot were probed with mouse monoclonal anti-Bcl-2 (Santa Cruz), anti-Bid, anti-caspase-8, anti-PARP(BD Pharmingen), and rabbit polyclonal anti-caspase-3 (Santa Cruz) antibodies. Immunoreactivity was detected using either and anti-mouse (Sigma) or anti-rabbit (Zymed) peroxidase-conjugated sencondary immunoglobulin G antibody followed by SuperSignal West Pico Chemiluminescent (PIERCE).

Results

1. Cytotoxic effect of CDST in lymphoma and leukemia cell lines

The effect of CDST on the cytotoxicity of cells was evaluated using the MTT assay. Shown as Fig. 2, a 24 h exposure to CDST dramatically decreased the viability of HL-60 cells in a dose-dependent manner, but other cell lines (U-937, Jurkat, Molt-4) was not represent almost cytotoxicity. The concentration required to inhibit growth of HL-60 cells by 50% (IC_{50}) was approximately 80 ㎍/㎖. These results suggest that CDST have cell-specific cytotoxicity about HL-60 cells.

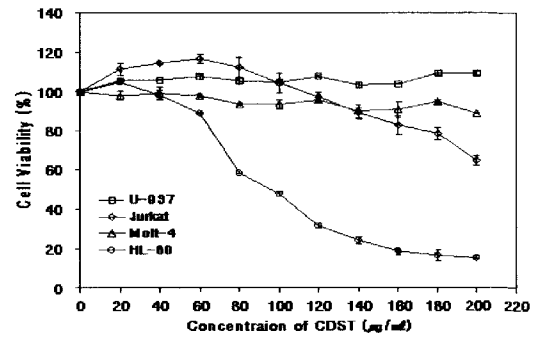

Fig. 2. Effect of CDST on cytotoxicity in lymphoma and lukemia cell lines. The cells (2×10^5 cells/㎖) were treated with various concentrations (20~200 ㎍/㎖) of CDST and the cells were tested for viability by MTT assay 24 h after the treatment of CDST. Value are means ± SD, N = 3.

2. Morphological changes in HL-60 cells treated with CDST

The morphology of cells treated with CDST was studied to test whether cell death was a result of apoptosis in HL-60

cells. Morphological characteristics of apoptosis, including nuclear condensation and fragmentation, were observed under fluorescent microscope by DAPI staining. The bright blue apoptotic nuclei were readily identified by their condensed chromatin and apoptotic bodies. HL-60 cells were treated with various concentration of CDST for 24 h or with concentration of 80 ㎍/㎖ CDST at certain time interval. In dose-dependent manner, CDST increased in the number of apoptotic cells from the concentration of 80 ㎍/㎖ in HL-60 cells (Fig. 3A) and in time-dependent manner, apoptotic cells was markedly detected after 12 h of incubation with CDST (Fig. 3B). Taken together, along with the appearance of elongated cells, disintegrated cells, as evidenced by apoptotic bodies, and cells with condensed nuclear chromatin appeared in a dose- and time-dependent manner in response to CDST treatment.

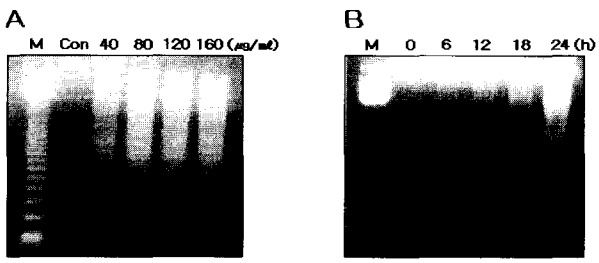

Fig. 3. Effect of CDST on DNA fragmentation in HL-60 cells. A dose-dependent of DNA fragmentation. (A) Dose-dependent manner of DNA fragmentation in HL-60 cells. The cells (2×10^5 cells/㎖) were incubated with 40, 80, 120, and 160 ㎍/㎖ of CDST for 24 h. (B) Time-dependent manner of DNA fragmentation in HL-60 cells. The cells were treated with 80 ㎍/㎖ of CDST for 0, 6, 12, 18, and 24 h. DNA was extracted, then separated by 1.2% agarose gel (contained ethidium bromide) electrophoresis, and visualized under UV light. M, 123bp DNA ladder marker Con, Control.

3. CDST induces apoptotic cell death in HL-60 cells

The biochemical hallmark of apoptosis in degradation of DNA by endogenous DNase, which cut the internucleosomal regions into double-stranded DNA fragments of 180~200 base pairs. We examined the effects of CDST on the internucleosomal DNA fragmentation in HL-60 cells treated with various concentration or certain time interval by agarose gel electrophoresis. A ladder pattern of internucleosomal DNA fragmentation was observed dose- and time-dependent when CDST was applied to the HL-60 cells (Fig. 4). As shown in Fig. 4, CDST induced DNA fragmentation at concentrations of 40~160 ㎍/㎖ (Fig. 4A) and the efficient induction for apoptosis was observed at 80 ㎍/㎖treatment for 18 h (Fig. 4B). These results suggest that CDST can trigger apoptosis of HL-60 cells.

4. CDST induces activation of caspase-3 and -8

Caspase family plays key roles in the execution of apoptotic cell death. Of these, caspase-3 and -8 is the most important cell executioners for apoptosis. We observed the proteolytic activation of procaspase-3 and -8 induced by the CDST. As shown in Fig 5, Western blot analysis showed that CDST-treatment induced time- and dose-dependent activation of caspase-3, and -8. CDST induced a nearly quantitative cleavage of procaspase-3 and -8 in 120 ㎍/㎖, 24 h and 80 ㎍/㎖, 12 h, respectively (Fig. 5). This result showed that CDST-induced caspase-8 activation can regulate caspase-3 directly or through cytochrome c release and the consequent caspase-9/caspase-3 activation. Activation of caspase-3 lead to the cleavage of a number of proteins, one of which is poly(ADP-ribose) polymerase (PARP). Although PARP is not essential for cell viability, the cleavage of PARP is another hallmark of apoptosis. Treatment of HL-60 cells with various concentrations or times of CDST induced dose- and time-dependent proteolytic cleavage of 116 kDa PARP with accumulation of the 89 kDa cleaved products (Fig. 5). These data suggest that CDST-induced apoptosis requires the activation of caspases, and CDST induce apoptotic cell death through caspase-8 activation.

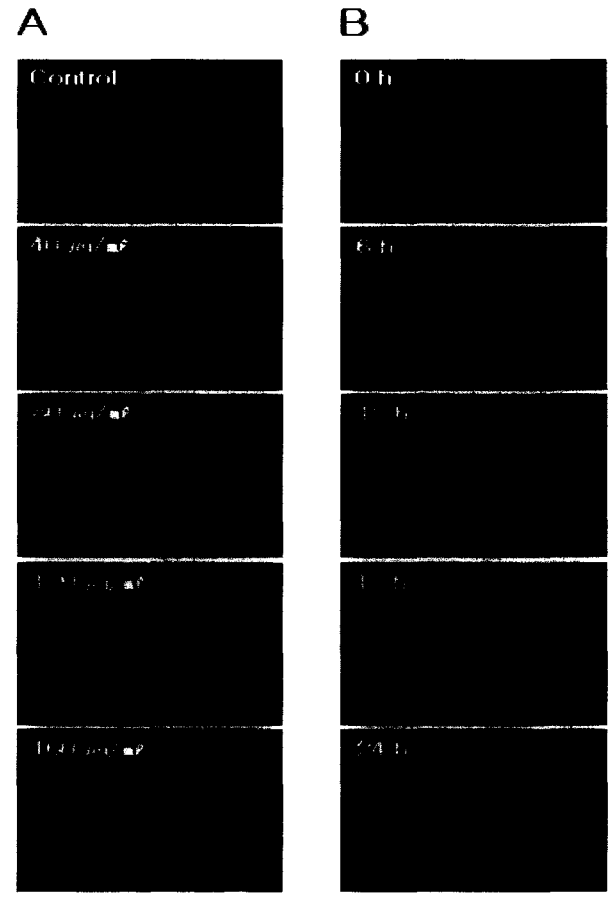

Fig. 4. Effect of CDST on HL-60 cells morphology. (A) Cells (2×10^5 cells/㎖) were treated with 40, 80, 120, and 160 ㎍/㎖ of CDST for 24 h. (B) Cells were treated with 80 ㎍/㎖ of CDST for 0, 6, 12, 18, and 24 h. After, cells were subjected to cytospin, stained with DAPI-methanol and observed under a fluorescence microscope. Chromatin condensation and apoptotic bodies stained bright blue.

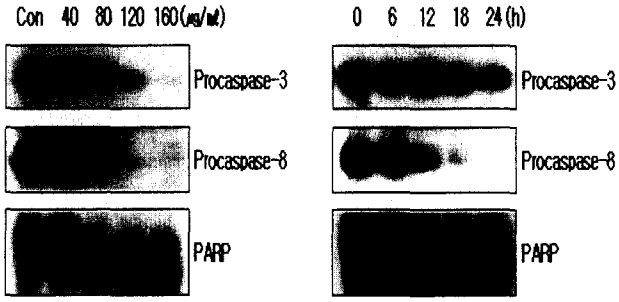

Fig. 5. Effect of CDST on activation of caspase-3, -8, and proteolytic cleavage of PARP in HL-60 cells. HL-60 cells (2×10^5 cells/㎖) were incubated with 40, 80 120, and 160 ㎍/㎖ CDST for 24 h or treated with CDST (80 ㎍/㎖) for 6, 12, 18, and 24 h. Whole cell lysates were subjected to SDS-PAGE followed by Western blotting with an anti-caspase-3 polyclonal, anti-caspase-8, and anti-PARP monoclonal antibodies.

5. CDST induces mitochondrial cytochrome c release through Bid cleavage and Bax translocation.

To analyze the involvement of cytochrome c release in CDST-induced apoptosis, cytosolic and mitochondrial fractions were obteined and analyzed for the presence of cytochrome c by Western blot analysis. It is known that cytochrome c released from mitochondria into the cytosol binds to apoptotic protease activating factor (Apaf) complex and triggers the activation of procaspase-9 to the active caspase-9. As shown in Fig. 6, CDST induced the release of cytochrome c in a dose- and time-dependent manner in the HL-60 cells. Fig. 6. showed that CDST treatment caused a time-dependent decrease in mitochondrial cytochrome c and a concomitant increase in cytosolic cytochorme c. It was clearly shown that CDST-induced apoptosis involves release of cytochrome c into the cytosol. Also, CDST-induced cytochrome c release was observed after caspase-8 activation. This is reflected that release of cytochrome c mediates by caspase-8 (Fig. 5 and 6). Induction of cytochrome c release from mitochondria occur via caspase-8-mediated cleavage of Bid and Bax mitochondrial translocation. The proapoptotic members of Bcl-2 family such as Bid and Bax play key roles in many drug-induced cytochrome c release. Also, Bid is one of the triggers for Bax translocation to mitochondria[41-45]. We analyzed Bid cleavage and Bax translocation by Western blot analysis. As shown in Fig. 6, cleavage of Bid and Bax translocation was observed from 80 ㎍/㎖, 12 h,and this corresponded with caspase-8 activation pattern. These data indicate that CDST depolarizes the potential of mitochondrial membranes via truncation of Bid, which stimulates the translocation of Bax to mitochondria, it follows that release of cytchorme c from mitochondria by occur via Bid cleavage and Bax translocation.We also studied the effect of CDST on Bcl-2. The antiapoptotic protein Bcl-2 is an integral membrane protein located mainly on the outer membrane of mitochondria[46] and to suppress the release of cytochrome c from mitochondria. However, the protein level of Bcl-2 was not changed by CDST treatment (Fig. 6.).

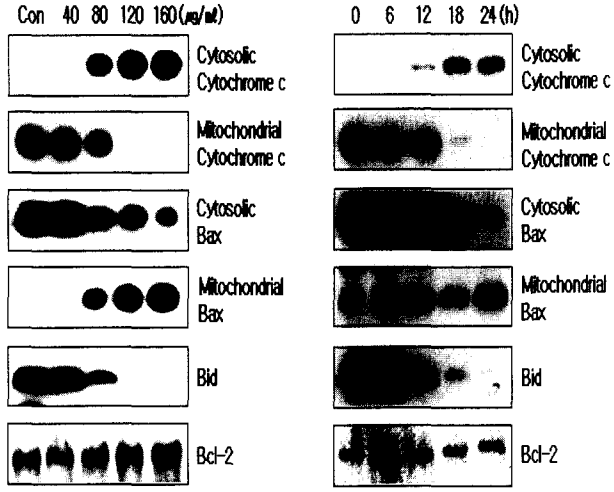

Fig. 6. Effect of CDST on Bcl-2, Bid cleavage, Bax translocation, and cytochrome c release in HL-60 cells. HL-60 cells (2×10^5 cells/㎖) were treated with various concentration (40~160 ㎍/㎖) of CDST for 24 h or treated with CDST (80 ㎍/㎖) for various time (0~24 h).Cytosolic and mitochondrial fractions were isolated as described under Materials and methods. Bcl-2, Bid, Bax, and Cytochrome c was determined by Western blot using anti-cytochrome c, anti-Bid monoclonal, anti-Bcl-2 and anti-Bax polyclonal antibodies.

Discussion

TIQ derivatives have been found to have various biological activities such as anticonvulsant, antihypertensive, antitumor, and antimalarial. The other TIQ derivatives variously synthesized as changed substitution group (hydroxyl, phosphonyl, methyl, ethyl, phenyl) in nitrogen of TIQ but TIQderivatives with nonpolor substitution group (methyl, ethyl, phenyl) not observed cytotoxicity at what kind of cancer cell lines too. Also, TIQ derivatives with polor substitution group (sulfamide, hydroxyl, phenyl) had not cytotoxicity at solid tumor cell lines (lung, breast, liver etc.), but only leukemia cell lines (Jurkat, Molt-4, HL-60) showed various cytotoxicity (Fig. 2). Above all things, CDST showed cell-specifically cytotoxic to human promyelocytic leukemia (HL-60) cells. These results indicate that CDST may have a possibility of potential chemotherapeutic agent for human promyelocytic leukemia disease. In the present report, we describe the mechanism through which CDST induces apoptosis of HL-60 cells. CDST activatedcaspase-8, induced Bid cleavage and Bax translocation, caused mitochondrial cytochrome c release, and induced caspase-3 activation and PARP cleavage in HL-60 cells but not in Bcl-2.

This is the first report to suggest that CDST activates caspase-8 and that it is required for apoptosis induced by

CDST, but how CDST activates caspase-8 is not clear. Because autoactivation induced by oligomerizationcan activate caspase-8[47,48], CDST may induce oligomerization of caspase-8. However, certain chemotherapeutic agents are known to induce apoptosis through induction of death receptors[49]. In the case of Fas/TNF-stimulated apoptotic signaling, cross-linking of Fas or TNF results in the recruitment of a set of proteins that include FADD/MORT1 and caspase-8 to the receptor forming the death-inducing signaling complex[50]. Once caspase-8 is recruited, it becomes activated automatically in the presence of FADD[51]. It is also possible that the TNF/Fas ligand system/receptor is involved in the activation of caspase-8 by CDST. We need further studies for caspase-8 activation by CDST in HL-60 cells.

Release of cytochrome c form mitochondria is a central event in apoptosis[52,53]. Cytochrome c release play a role in amplifying the effects of caspase-8[54]. Caspase-8 can activate apoptotic pathways involving effector caspases through mitochondria-dependent or -independent pathways. In the mitochondria-independent pathway, caspase-8 can activate caspases-3 directly, but in the mithochondria-dependent, which involves the release of cytochrome c from mitochondria, activate caspase-3 through induction of caspase-9. Direct activation of caspase-3 by caspase-8 involvedwhen caspase-8 is present at a high concentration. However, when caspase-8 is present at a low concentration, caspase-8 required the mitochondria to complete engagement of the apoptotic machinery, and the effect of low concentration of caspase-8 was vastly amplified through cytochrome c-dependent caspase activation[54,55]. Our results show that the activity of caspase-8 by CDST was lower than that of caspase-3 (Fig 5), and also caspase-8 induced the release of cytochrome c from mitochondria through Bid cleavage and Bax translocation (Fig. 6). It suggest that CDST-induced apoptosis involve mitochondria-dependent pathway through activation of caspase-8. But, It is unclear how caspase-8 can overcome the inhibitory effects of Bcl-2 on cytochrome c release, Because CDST has no effect on level of Bcl-2 protein. Srivastava et al.[56] reported that antiapoptotic activity of Bcl-2 inhibited by JNK-mediated phosphorylation. Assumedly, CDST may induce phosphorylation of Bcl-2.

Also, CDST can induce cleavage of the proapototic protein Bid (Fig. 6). Bid exists in the cytosolic fraction of living cells as an inactive precursorthat becomes activated upon cleavage by caspase-8. After cleavage, Bid translocates onto mitochondria. The actived Bid by itself is sufficient to induce complete release of cytochrome c from mitochondria. In this study we have shown that Bax mediates mitochondrial cytochrome c release during CDST-induced apoptosis (Fig. 6). Bax is another proapoptotic member of the Bcl-2 family of proteins. Bax translocates from its predominantly cytoplasmic location to the mitochondria upon apoptosis induction. However, Bid is much more potent cytochrome c releasing factor than Bax. Bax only release up to 20% of the total mitochondrial cytochrome c even at a high concentration[58]. Also, cleaved Bid is reported to be more efficient for triggering the oligomerization and translocation of Bax into mitochondrial membrane[41-45].

In conculsion, CDST mediates apoptosis of HL-60 cells through caspase-8 which involves cleavage of Bid and translocation of Bax to mitochondria where it promotes the release of cytochrome c, but not changed in Bcl-2. Our results contribute to the ordering of events druing CDST-induced apoptosis, by demonstrating that caspase-8 is responsible for cytochrome c release and caspase-3 activation. Further studies will be required to identify the specific signals that induce caspase-8 activation by CDST.

Acknowledgment

This study was supported by a grant of the Oriental Medicine R&D Project (02-PJ9-PG1-CO05-0003), Ministry of Health & Welfare, Republic of Korea.

References

1. Anan, H., Tanaka, A., Tsuzuki, R., Yokota, M., Yatsu, T. and Fujikura, T., 4-(3,4-Dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline derivatives. II. Their renal vasodilation activity and structure-activity relationship. Chem. Pharm. Bull. (Tokyo), 44, 1865-1870, 1996.
2. Chau, P., Czuba, I.R., Rizzacasa, M.A, Bringmann, G., Gulden, K.P. and Schaffer, M. Convergent Synthesis of Naphthylisoquinoline Alkaloids: Total Synthesis of (+)-O-Methylancistrocline. J. Org. Chem., 61, 7101-7105, 1996.
3. Corey, E.J. and Gim D.Y., A convergent enantioselective synthesis of the tetrahydroisoquinoline unit in the spiro ring of ecteinascidin 743. Tetrahedron Lett., 37, 7163-7166, 1996.
4. Dong, H., Lee, C.M., Huang, W.L. and Peng, S.X., Cardiovascular effects of substituted tetrahydroisoquinolines in rats. Br. J. Pharmacol., 107, 262-268, 1992.
5. Grunewald, G.L., Dahanukar, V.H., Caldwell, T.M. and Criscione, K.R., Examination of the role of the acidic hydrogen in imparting selectivity of 7-(aminosulfonyl)-1,2,3,4- tetrahydroisoquinoline (SK&F 29661) toward inhibition of henylethanolamine

N-methyltransferase vs the alpha 2-adrenoceptor. J. Med. Chem., 40, 3997-4005, 1997.

6. Hallocka, Y.F., Cardellina, II, Kornekb, T., Guldenb, K.P., Bringmann, G. and Boyd, M.R., Gentrymine B1, the first quaternary isoquinoline alkaloid from Ancistrocladus korupensis. Tetrahedron Lett., 36, 4753-4756, 1995.
7. Hoye, T.R. and Mi, L., Total syntheses of korupensamine C and ancistrobrevine B. Tetrahedron Lett., 37, 3097-3098, 1996.
8. McNaught, K.S., Thull, U., Carrupt, P.A., Altomare, C., Cellamare, S., Carotti, A., Testa, B., Jenner, P. and Marsden, C. D., Inhibition of complex I by isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Biochem. Pharmacol., 50, 1903-1911, 1995.
9. McNaught, K.S., Thull, U., Carrupt, P.A., Altomare, C., Cellamare, S., Carotti, A., Testa, B., Jenner, P. and Marsden, C. D., Inhibition of [3H]dopamine uptake into striatal synaptosomes by isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Biochem. Pharmacol., 52, 29-34, 1996.
10. Nakagawa, H., Makino, Y., Yoshida, Y., Ohta, S. and Hirobe, M., A novel metabolite of 3,4-methylenedioxyamphetamine (MDA): formation of 3-methyl-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline and its pharmacological effect. Biol. Pharm. Bull., 16, 579-82, 1993.
11. Ohkubo, M., Kuno, A., Katsuta, K., Ueda, Y., Shirakawa, K., Nakanishi, H., Nakanishi, I., Kinoshita, T.and Takasugi, H., Studies on cerebral protective agents. IX. Synthesis of novel 1,2,3,4-tetrahydroisoquinolines as N-methyl-D-aspartate antagonists. Chem. Pharm. Bull. (Tokyo), 44, 95-102, 1996.
12. Salvadori, S., Balboni, G., Guerrini, R., Tomatis, R., Bianchi, C., Bryant, S. D., Cooper, P. S. and Lazarus, L. H., Evolution of the Dmt-Tic pharmacophore: N-terminal methylated derivatives with extraordinary delta opioid antagonist activity. J. Med. Chem., 40, 3100-3108, 1997.
13. Scott, J.D. and Williams, R.M., Chemistry and biology of the tetrahydroisoquinoline antitumor antibiotics. Chem. Rev., 102, 1669-1730, 2002.
14. Shirahata, A., Yoshioka, M., Tamura, Z., Fluorometric determination of 1,2,3,4-tetrahydro-6,7-dihydroxyisoquinoline in biological materials by HPLC. Chem. Pharm. Bull. (Tokyo), 45, 1814-1819, 1997.
15. VanAtten, M.K., Ensinger, C.L., Chiu, A.T., McCall, D.E., Nguyen, T.T., Wexler, R.R. and Timmermans, P.B., A novel series of selective, non-peptide inhibitors of angiotensin II binding to the AT2 site. J. Med. Chem., 36, 3985-3992, 1993.
16. Whaley, W. M. and Govindachari T. R., The Preparation of 3,4-Dihydroisoquinolines and Related Compounds by the Bischler-Napieralski Reaction. Org. React., 6, 74-206, 1951.
17. Kalechman, Y., Longo, D. L., Catane, R., Shani, A., Albeck, M. and Sredni, B. Synergistic anti-tumoral effect of paclitaxel (Taxol)+AS101 in a murine model of B16 melanoma: association with ras-dependent signal-transduction pathways. Int J Cancer. 86, 281-288, 2000.
18. Gamet-Payrastre, L., Li, P., Lumeau, S., Cassar, G., Dupont, M.A., Chevolleau, S., Gasc, N., Tulliez, J. and Terce, F. Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. Cancer Res. 60, 1426-1433, 2000.
19. Ling, Y.H., Yang, Y., Tornos, C., Singh, B. and Perez-Soler, R. Paclitaxel-induced apoptosis is associated with expression and activation of c-Mos gene product in human ovarian carcinoma SKOV3 cells. Cancer Res. 58, 3633-3640, 1998.
20. Nieves-Neira, W. and Pommier, Y. Apoptotic response to camptothecin and 7-hydroxystaurosporine (UCN-01) in the 8 human breast cancer cell lines of the NCI Anticancer Drug Screen: multifactorial relationships with topoisomerase I, protein kinase C, Bcl-2, p53, MDM-2 and caspase pathways. nt J Cancer. 82, 396-404, 1999.
21. Piazza, G.A., Rahm, A.L., Krutzsch, M., Sperl, G., Paranka, N.S., Gross, P.H., Brendel, K., Burt, R.W., Alberts, D.S. Pamukcu, R. and et al. Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. Cancer Res. 55, 3110-3116, 1995.
22. Evan, G. and Littlewood, T. A matter of life and cell death. Science. 281, 1317-1322, 1998.
23. Thornberry, N.A. and Lazebnik, Y. Caspases: enemies within. Science. 281, 1312-1316, 1998.
24. Piao, W., Yoo, J., Lee, D.K., Hwang, H.J. and Kim, J.H. Induction of G(2)/M phase arrest and apoptosis by a new synthetic anti-cancer agent, DW2282, in promyelocytic leukemia (HL-60) cells. Biochem Pharmacol. 62, 1439-1447, 2001.
25. Kim, R., Tanabe, K., Uchida, Y., Emi, M., Inoue, H. and Toge, T. Current status of the molecular mechanisms of anticancer drug-induced apoptosis. The contribution of molecular-level analysis to cancer chemotherapy. Cancer Chemother Pharmacol. 50, 343-352, 2002.
26. Ashkenazi, A. and Dixit, V.M. Apoptosis control by death and decoy receptors. Curr. Opin. Cell Biol., 11, 255-260, 1999.
27. Sun, X.M., MacFarlane, M., Zhuang, J, Wolf, B.B., Green, D.R. and Cohen, G.M. Distinct caspase cascades are initiated in receptor-mediated and chemical-induced apoptosis. J Biol Chem. 274, 5053-5060, 1999.
28. Green, D.R. Apoptotic pathways: the roads to ruin. Cell. 94, 695-698, 1998.

29. Kroemer, G., Zamzami, N. and Susin, S.A. Mitochondrial control of apoptosis. Immunol Today. 18, 44-51, 1997.
30. Mignotte, B. and Vayssiere, J.L. Mitochondria and apoptosis. Eur J Biochem. 252, 1-15, 1998.
31. Zou, H., Li, Y., Liu, X. and Wang, X. An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J Biol Chem. 274, 11549-11556, 1999.
32. Luo, X., Budihardjo, I., Zou, H., Slaughter, C. and Wang, X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell. 94, 481-490, 1998.
33. Li, H., Zhu, H., Xu, C. J. and Yuan, J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. 94, 491-501, 1998.
34. Finucane, D.M., Bossy-Wetzel, E., Waterhouse, N.J., Cotter, T. G. and Green, D. R. Bax-induced caspase activation and apoptosis via cytochrome c release from mitochondria is inhibitable by Bcl-xL. J Biol Chem. 274, 2225-2233, 1999.
35. Murphy, K.M., Streips, U.N. and Lock, R.B. Bax membrane insertion during Fas(CD95)-induced apoptosis precedes cytochrome c release and is inhibited by Bcl-2. Oncogene. 18, 5991-5999, 1999.
36. Kluck, R.M., Bossy-Wetzel, E., Green, D.R. and Newmeyer, D. D. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science. 275, 1132-1136, 1997.
37. Gross, A., McDonnell, J.M. and Korsmeyer. S.J. BCL-2 family members and the mitochondria in apoptosis. Genes Dev. 13, 1899-1911, 1999.
38. Hsu, Y.T. and Youle, R.J. Bax in murine thymus is a soluble monomeric protein that displays differential detergent-induced conformations. J Biol Chem. 273, 10777-10783, 1998.
39. Finucane, D.M., Bossy-Wetzel, E., Waterhouse, N.J., Cotter, T.G., Green, D.R. Bax-induced caspase activation and apoptosis via cytochrome c release from mitochondria is inhibitable by Bcl-xL. J Biol Chem. 274, 2225-2233, 1999.
40. Pae, H.O., Oh, H., Yun, Y.G., Oh, G.S., Jang, S.I., Hwang, K. M., Kwon, T.O., Lee, H.S. and Chung, H.T. Imperatorin, a furanocoumarin from Angelica dahurica (Umbelliferae), induces cytochrome c-dependent apoptosis in human promyelocytic leukaemia, HL-60 Cells. Pharmacol Toxicol. 91, 40-48, 2002.
41. Luo, X., Budihardjo, I., Zou, H., Slaughter, C. and Wang, X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell. 94, 481-490, 1998.
42. Desagher, S., Osen-Sand, A., Nichols, A., Eskes, R., Montessuit, S., Lauper, S., Maundrell, K., Antonsson, B. and Martinou, J.C. Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. J Cell Biol. 144, 891-901, 1999.
43. Eskes, R., Desagher, S., Antonsson, B. and Martinou, J.C. Bid induces the oligomerization and insertion of Bax into the outer mitochondrial membrane. Mol Cell Biol. 20, 929-935, 2000.
44. Korsmeyer, S.J., Wei, M.C., Saito, M., Weiler, S., Oh, K.J. and Schlesinger, P.H. Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. Cell Death Differ. 7, 1166-1173, 2000.
45. Ruffolo, S.C., Breckenridge, D.G., Nguyen, M., Goping, I.S., Gross, A., Korsmeyer, S.J., Li, H., Yuan, J. and Shore, G.C. BID-dependent and BID-independent pathways for BAX insertion into mitochondria. Cell Death Differ. 7, 1101-1108, 2000.
46. Hockenbery, D., Nunez, G., Milliman, C., Schreiber, R.D. and Korsmeyer, S.J. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature. 348, 334-336, 1990.
47. Muzio, M., Stockwell, B.R., Stennicke, H.R., Salvesen, G.S. and Dixit, V. M. An induced proximity model for caspase-8 activation. J Biol Chem. 273, 2926-2930, 1998.
48. Srinivasula, S.M., Ahmad, M., Fernandes-Alnemri, T. and Alnemri, E.S. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. Mol Cell. 1, 949-957, 1998.
49. Muller, M., Strand, S., Hug, H., Heinemann, E.M., Walczak, H., Hofmann, W.J., Stremmel, W., Krammer, P.H. and Galle, P. R. Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. J Clin Invest. 99, 403-413, 1997.
50. Muzio, M., Chinnaiyan, A.M., Kischkel, F.C., O'Rourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J.D., Zhang, M., Gentz, R., Mann, M., Krammer, P.H., Peter, M.E. and Dixit, V. M. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death--inducing signaling complex. Cell. 85, 817-827, 1996.
51. Medema, J.P., Scaffidi, C., Kischkel, F.C., Shevchenko, A., Mann, M., Krammer, P.H., Peter, M.E. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J. 16, 2794-2804, 1997.
52. Liu, X., Kim, C.N., Yang, J., Jemmerson, R., Wang, X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell. 86, 147-157, 1996.
53. Reed, J.C. Cytochrome c: can't live with it-can't live without it. Cell. 91, 559-562, 1997.
54. Kuwana, T., Smith, J.J., Muzio, M., Dixit, V., Newmeyer, D. D. and Kornbluth, S. Apoptosis induction by caspase-8 is

amplified through the mitochondrial release of cytochrome c. J Biol Chem. 273, 16589-16594, 1998.

55. Zhuang, S., Lynch, M.C. and Kochevar, I.E. Caspase-8 mediates caspase-3 activation and cytochrome c release during singlet oxygen-induced apoptosis of HL-60 cells. Exp Cell Res. 250, 203-212, 1999.
56. Srivastava, R.K., Mi, Q.S., Hardwick, J.M. and Longo, D.L. Deletion of the loop region of Bcl-2 completely blocks paclitaxel-induced apoptosis. Proc Natl Acad Sci U S A. 96, 3775-3780, 1999.
57. Jurgensmeier, J.M., Xie, Z., Deveraux, Q., Ellerby, L., Bredesen, D., Reed, J.C. Bax directly induces release of cytochrome c from isolated mitochondria. Proc Natl Acad Sci U S A. 95, 4997-5002, 1998.