Involvement of apoptotic signals in cyclosporin A-induced proliferation of human gingival fibroblast

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Ⅰ. Introduction

Cyclosporin A(CsA) has been widely used as an immunosuppressive drug to prevent graft rejection after organ transplantation and to treat several autoimmune disorders such as rhumat arthritis atropic dermatitis, psoriasis an Behcet's disease[1]. Unfortunately, clinical use of CsA is often associated with side effects including hepatoxicity, nephrotoxicity, neurotoxicity, hypertension and gingival overgrowth[2,3]. The occurrence of CsA-induced gingival overgrowth(CsAGO) is highly variable, but regularly appears in more than 70% of adult transplant recipient[4]. CsAGO may interfere with normal physiologic function such as mastication and speech and may also have a oral hygiene problem and a psychological impact[5].

Despite extensive studies over the decades, pathogenesis of CsAGO remains still unsettled. There has been much controversy in pathogenesis of CsAGO with conflicting evidences as to whether it represents a true hyperplasia. Some previous studies have demonstrated that CsA and its major metabolites OL-17 react with a distinct subpopulation of gingival fibroblast, causing an increments in protein synthesis and rate of cell proliferation[6-8]. Furthermore, these effects of CsA on human fibroblast proliferation may vary according to individual cell strains[9]. In contrast, other previous studies have suggested that CsA increases production of extracellular matrix, collagen and interleukin-6 but simultaneously decreases proliferation of human gingival fibroblast[10,11]. Nevertheless, it is apparent that CsA increases cell proliferation of human gingival fibroblast in vitro[12].

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Recent previous reports have suggested that various growth factors may involve in cell proliferation promoted by CsA in human gingival fibroblast [12-14]. In addition, there is another possibility that antiapoptotic signals and its regulating molecules may be involved in enhanced proliferation by CsA in human gingival fibroblast since cell survival rate depends on balance between proliferation and cell death. In practice, overexpression of Bcl-2, an antiapoptotic molecule, promotes myocyte proliferation and upregulation of Bcl-2 enhances proliferation of vascular endothelial cell, suggesting the possibility that antiapoptotic signals may involve in enhanced proliferation by CsA in human gingival fibroblast. Besides, Bcl-2 promotes regeneration of severed axon in mammalian CNS[15]. Up to date, however, the roles of antiapoptotic signals and its regulating molecules have been not studied in CsA-induced cell proliferation of human gingival fibroblast in vitro.

On the other hand, Apoptosis is driven from the activation of a family of cysteine protease called caspases, which then cleave a critical set of cellular proteins to initiate apoptotic cell death [16,17]. These family are expressed as proenzymes and are activated by upstream stimuli. Among mammalian caspases of at least 14 known members, those involved with apoptosis can be further subdivided into the initiator caspases(-2, -8, -9 and -10) and the effector caspases(-3, -6 and -7) [18,19].

Two main pathways of activating caspases are death receptor-mediated mechanism and mitochondria-mediated mechanism. Both pathways share the activation of caspase-3 as an executioner caspase, which activates caspase-activated DNase, causing apoptotic DNA fragmentation. Death receptor pathway is stimulated by cell surface death receptors such as tumor necrosis factor (TNF) receptor and Fas [20]. The receptors activated by ligands lead to caspase-8 activation, with subsequent activation of caspase-3. The mitochondrial pathway is initiated from release of cytochrome *c* from mitochondria into cytosol, subsequently resulting in caspase-9 activation which causes the activation of caspase-3.

In addition to the caspase, members of the Bcl-2 protein family are also critical for the regulation of apoptosis. Bcl-2 family control the release of mitochondrial cytochrome *c* by regulating the permeability of the outer mitochondrial membrane. Bcl-2 family members are functionally divided into antiapoptotic molecules(Bcl-2, Bcl-X_L, Bcl-W, Mcl-1, A1) and proapoptotic molecules (Bax, Bcl-1s, Bid, Bad, Bim, Bik) [18,19]. Among the Bcl-2 protein family, Bcl-2 and Bcl-X_L are prominent antiapoptotic family whereas Bax, Bid and Bak are prominent proapoptotic family [15].

Based on the possibility that antiapoptotic signals may be involved in CsAGO, the present study was designed to investigate the roles of molecules associated with mitochondria- and death receptor-mediated apoptotic signals in CsA-induced cell proliferation in human gingival fibroblasts.

Ⅱ. Materials and methods

1. Cell culture and cell viability assay

Human gingival fibroblast(HGF) cells were obtained from heathy gingival tissue of patient in Chonnam National Hospital. HGF cell were maintained in DMEM media supplemented with 10% fetal bovine serum (Gibco, USA) under 5% CO_2 at 37℃. Cyclosporin A (CsA, Sigma, USA) was dissolved in distilled DMEM and sterilized through 0.2 ㎛ filter. Cell viability was determined using MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide) assay(Sigma, USA).

2. Observatoion of cell morphology by microscope

Cells were plated on 8-well chamber slide at a density of 1×10^4 was incubated for 18 h, subsequently followed by treatment CsA was treated with different concentrations(1, 5, 10, 50μM). Cell proliferation by CsA was obervated under microscope(Olympus, USA).

3. Detection of ROS production and caspase activity

Reactive oxygen species(ROS) production was monitered by fluorescence spectrometer (Hitachi F-4500, Japan) using 2′, 7′-dichlorofluor-bescin diacetate(DCF-DA). Cells were plated on 96-well plate and treated with N-acetyl-cysteine(NAC: Sigma, USA) and CsA. DCF-DA(25 μM) was added into the media for further 10 min at 37℃. Excitation was measured at 480 nm wavelength and emission was measured at 530 nm. Caspase activities were assessed by ELISA reader using the caspase-3, -9 activity assay kit (Calbiochem, CA) and caspase-8 activity kit (Santa Cruz, USA) according to the manufacturer's instructions.

4. Isolation of total RNA and reverse transcription polymerase chain reaction(RT-PCR)

For extraction of total RNA, cells were homogenized with a polytron homogenizer in Trizol reagent(Gibco-BRL, USA). RNA samples were quantified by spectrophotometry at 260 nm wavelength. For synthesis of c-DNA, 2 ㎍ of total RNA and 2 ㎕ of Oligo-dT (10pmoles) were mixed with 50 ㎕ RNase-free water, and then incubated at 42°C for 1 h and 94℃ for 5 min. PCR products were generated in PCR buffer containing 10 pmoles of each primer using PCR-premix kit (Bioneer, Korea). After the first denaturation step(5 min at 95℃), samples were subjected to 30 cycles consisting of 40 sec at 95℃, 40 sec at 55℃, and 1 min 30 sec at 72℃, with a final extention step of 10 min, on a GeneAmp PCR system(Perkin-Elmer 2400). The following primer pairs were used: for Fas, 5′-CAAGGGACTGATAGCATCTTTGAGG-3′(sense), 5′-TCCAGATTCAGGGTCACAGGTTG-3′(anti-sense), for VDAC 1, 5′-TGATACC ACGTTA GACCTCC-3′(sense), 5′-ACAACCTGGAAGCTATTTCA-3′(anti sense), for VDAC 2, 5′-TGCAGTGGTGTGGAATTTT-3′(sense), 5′-ACGAGTGCAGTTGGTACCTGA-3′(antisense), for VDAC 3,5′-

GCTGCTAAGTATAGGCTGGA-3′(sense),5′-CCACTGGATGGATCTGTAAT-3′(antisense), for Bcl-2, 5′-ACTTTGCAGAGATGTCCAGT-3′(sense), 5′-CGGTTCAGGTACTCAGTCAT-3′(antisense), for Bax, 5′-TTTGTTCAGGGTTTCATCC-3′(sense), 5′-ATCTTCTTCCAGATGGTGAG-3′(antisense). The amplified products were analyzed on 1.5% agarose gels containing ethidium bromide and visualized by UVP Transilluminator/ Polaroid camera System(UVP Laboratories, CA). RT-PCR was performed with primers for the housekeeping gene, GAPDH, as a control. The following primer pairs for GAP- DH were used: 5′-TGCATCCTGCACCACCAACT-3′(sense primer) and 5′-CGCCTGCTTCACCACTTC-3′(antisense primer). The intensities of the obtained bands were determined using the NIH Scion Image Software.

5. Western blotting

Cells were washed twice with PBS and proteins solubilized in the lysis buffer(1% NP-40, 500 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM Benzamid, 1 μg /ml Trypsin inhibitor) containing a cocktail of protease inhibitor(Complete, Germany). To determinate cytosolic cytochrome c, pellet was resuspended in extraction buffer containing 220 mM mannitol, 68 mM sucrose, 50 mM PIPES-NaOH(pH 7.4), 50 mM Kcl, 5 mM EGTA, 2 mM MgCl2, and 1 mM DTT. Lysates were incubated for 30 min at 4℃, centrifuged at 11000 x g for 20 min and protein concentrations were determined by BCA protein assay(Pierce, IL). Protein extracts(100~300 μg) were boiled for 5 min with SDS-sample buffer and then subjected to electrophoresis on 12% polyacrylamide gel. Proteins were electroblotted onto nitrocellulose membrane(Amersham Pharmacia Biotech, UK) and blocked with 5% skim milk(Becton Dickinson, USA) in Tris-buffered saline-0.1% Tween 20(TBS-T). Primary antibodies used were a rat monoclonal anti-cytochrome c(Pharmingen, CA), Bax, Bcl-2 and Bid(Santa Cruz, USA) were applied. Blots were subsequently washed three times in TBS-T for 5 min and incubated with specific peroxidase-coupled secondary antibodies(Sigma, USA). Bound antibodies were visualized using an enhanced chemiluminescent detection system (Amersham Pharmacia Biotech, UK).

Ⅲ. Results

1. CsA promotes proliferation and reduces cytosolic ROS level in HGF cells

The effects of CsA on the proliferation of HGF were assessed by MTT assay. As shown in Figure 1 and 2, cell viability was gradually enhanced in a dose-dependent manner when HGF cells were exposed to 0.1~50 μM CsA(Figure 1, 2). CsA increased HGF proliferation about 120~160% relative to control over 0.1~10 μM, CsA concentration used in this experiment is similar to the plasma level(100-200 μg/ml) in patients undergoing CsA-treatment. CsA showed maximal proliferation rate at 5 μM and CsA induced cell death of HGF above 50 μM. 5 μ

M CsA promoted HGF proliferation in a time- dependent manner. To determine whether CsA reduces cytosolic ROS level in HGF or not, cytosolic ROS was measured in CsA- treated HGF using DCF-DA. Figure 3 showed that 5 μM CsA reduced cytosolic ROS level in the HGF cells.

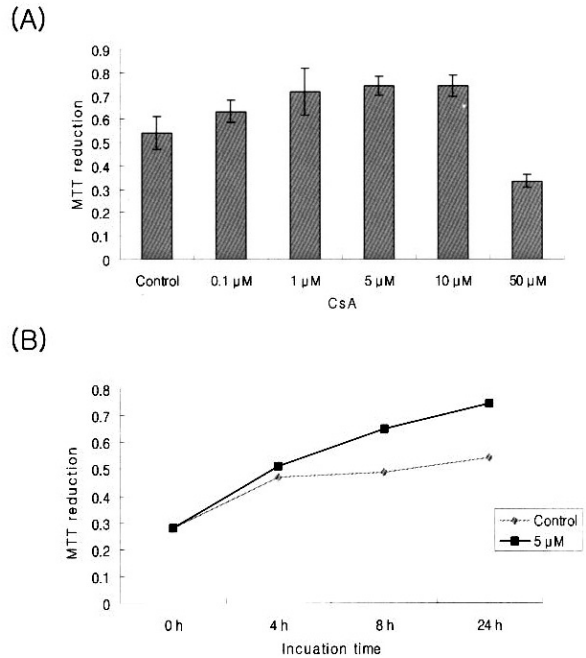

Figure 1. Effects of CsA on proliferation in HGF cells. Cell viability was determined by MTT assay as described in materials and methods. HGF cells were incubated with CsA for 24 h (A) and 5 μM CsA for indicated times (B). The viability increased in a dose- and time-dependent manner over 0.1~10 μM in CsA -treated HGF cells, whereas it decreased above 50 μM CsA-treated cells.(Data are mean±SD from 5 independent experiments.)

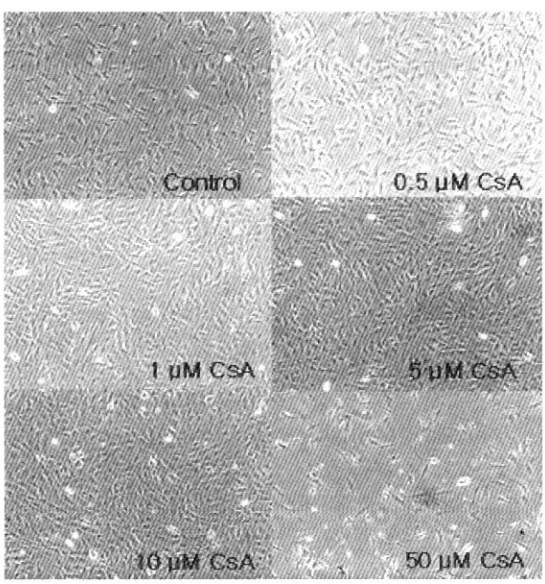

Figure. 2. CsA induced cell proliferation of HGF cells. HGF cells were incubated with different concentration(0.5, 1, 5, 10, 50μM) of CsA for 12h. Cell morphology was observed by microscope(x200). CsA enhanced proliferation of HGF cells in a dose-dependent manner over 0.1~10 μM , while it induced cell death above 50 μM CsA.

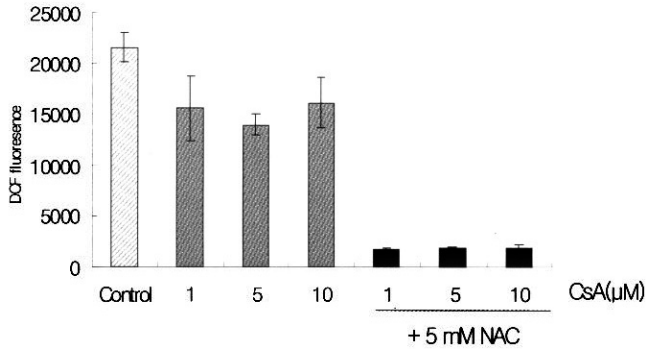

Figure. 3. Cytosolic ROS level was reduced CsA-treated HGF cells. HGF cells loading DCF was incubated for 12 h with different concentration(1, 5, 10 μM) of CsA alone or coincubation with 5 mM N-acetyl-C-cysteine(NAC) for 1 h. The intracellular levels of ROS were detected by measuring the DCF-DA fluorescence. CsA diminished cytosolic ROS level in HGF cells and NAC, free radical scavenger, greatly decreased the cytosolic ROS(Data are mean±SD from 5 independent experiments).

2. Bax and Bid is downregulated versus Bcl-2 is upregulated in CsA-treated HGF apoptosis

Generally, Bax, Bid and Bcl-2 has proven to be significant for cell survival determination, since Bax and Bid, cytochrome c releasers from mitochodrial, lead to apoptotic cell death and Bcl-2, a inhibitor of cytochrome *c* release from mitochondira,

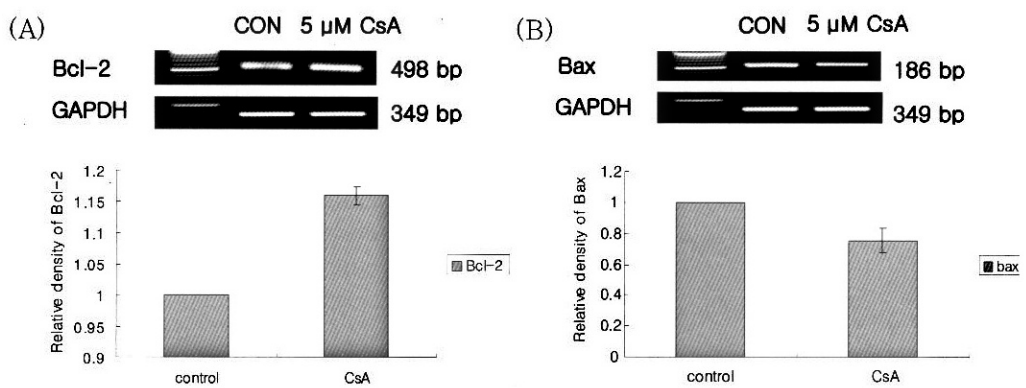

Figure. 4. Altered mRNA expression of Bcl-2 and Bax in CsA-treated HGF cells. After incubation of HGF cells with 5 μM CsA for 12 h, mRNA levels of Bcl-2 and Bax were determined using RT-PCR analysis. CsA upregulated Bcl-2 expression in mRNA levels. Whereas it downregulated Bax expression CsA-treated HGF. The histograms showed the relative density of Bcl-2 and Bax expression, respectively, relative to control(Values are mean±SD from 5 independent experiments).

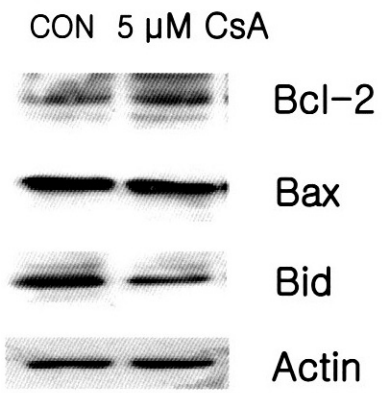

Figure. 5. Altered protein expression of Bcl-2 family in CsA-treated HGF cells. After incubation of HGF cells with 5 μM CsA for 12 h, protein leves of Bcl-2 family were determined using western blot analysis. Bcl-2 protein was upregulated, while Bax protein was downregulated in CsA-treated HGF cells.

leads to antiapoptotic effect. After the treatment of HGF cells with 5 μM CsA for 8 h, expression level of Bax, Bid and Bcl-2 in HGF cells were determined using RT-PCR and western blot. Figre 4 and 5 showed that CsA upregulated Bcl-2 expression and downregulated the expression of Bax and Bid.

3. Mitochondria is associated with CsA-induced HGF proliferation

To investigate whether mitochondria are involved in CsA-induced proliferation of HGF cells, the amount of cytochrome *c* released from mitochondira into cytosol was determined using western blot in cytosolic fractions as previous method. Cytosolic level of cytochrome *c* was assumed as a consequence of cytochrome *c* released from mitochondria into cytosol. Cytosolic cytochrome *c* was diminished in dose-dependent manner in response to exposure of CsA for 8 h, even if cytochrome *c* was slightly enhanced at 0.5 and 1 μM CsA(Figure 6A). Effects of CsA on voltage-dependent anion channel(VDAC) was determined using RT-PCR in order to elucidate the mechanism by which CsA inhibit cytochrome c release from mitochondria into cytosol. Expression of VDAC 1 and 3 were downregulated but VDAC 2 expression was not changed in CsA-treated HGF cells(Figure 6B). From these results, it is speculated that mitochondria-mediated apoptotic signals may be involved in CsA-induced proliferation of HGF cells.

4. Caspases are involved in CsA-induced proliferation in HGF cells

Caspases activities were measured on the basis that active caspases consequently cleave their substrate at a specific site. LEHD-pNA(200 μM), and DEVD-pNA(200 μM) were used as substrates for caspase-9, and -3 respectively. Caspase-9 and -3 activities was reduced relative to control in response to 1~10 ㎛ CsA for 8 h in HGF cells(Figure 7A, 7B).

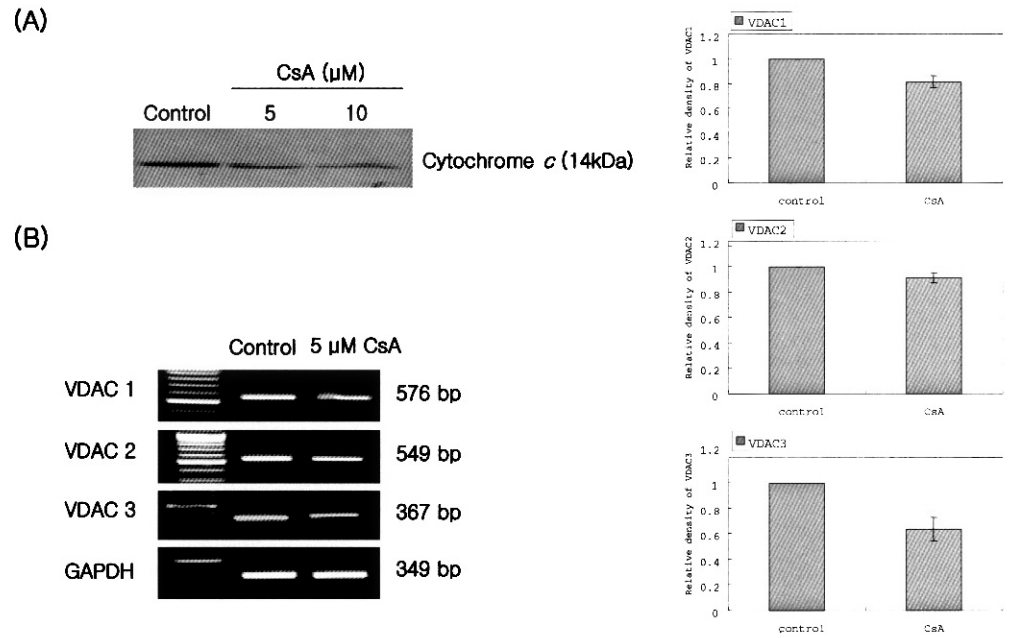

Figure 6. Decreased release of cytochrome c from mitochondria into cytosol and down-regulated expression of VDAC in CsA-treated HGF cells. After HGF cells exposed to CsA with different concentration for 12 h, cytosolic cytochrome *c* were determined using western blot analysis. CsA decreased the release of cytochrome c from mitochondria into cytosol in HGF cells (A). After incubation of HGF cells with 5 μM CsA for 12 h, mRNA levels of VDAC were determined using RT-PCR analysis. CsA downregulated expression of VDAC 1 and 3, but VDAC 2 expression was significantly not affected (B). (Values are mean±SD from 5 independent experiments).

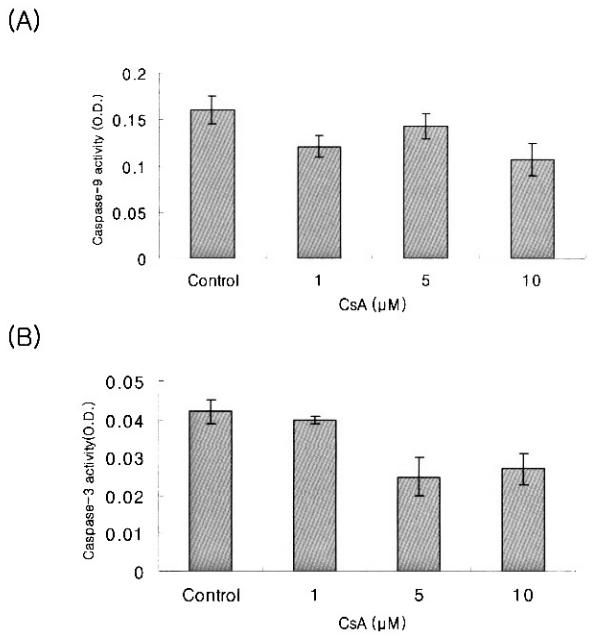

Figure 7. Effects of CsA on activity of caspase-9 and -3 in CsA -treated HGF cells. Cells was treated with different concentration of CsA(1, 5, 10 μM) for 1 h. Absorbance for caspases was measured in the wells at 405 nm by ELISA reader as described in materials and methods. CsA decreased activity of caspase-9 (A) and caspase-3 (B), respectively. (Results are mean±SD from 5 experiments).

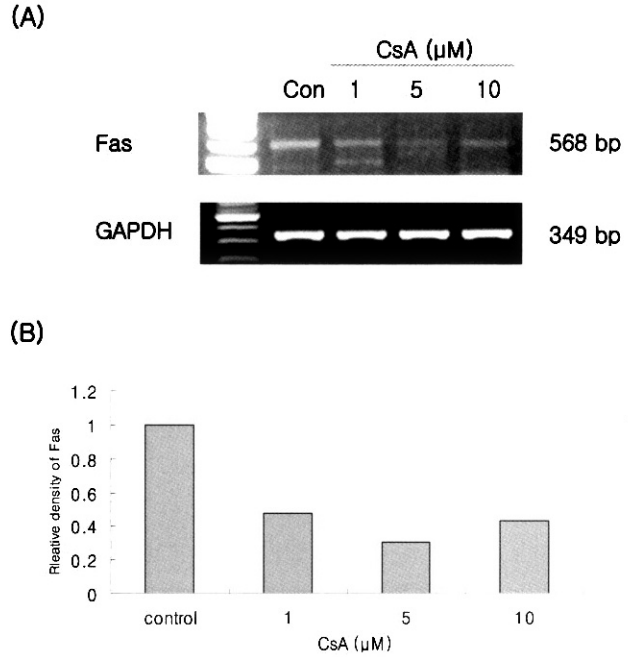

Figure 8. Downregulated Fas and caspase-8 in CsA-treated HGF cells. After incubation of HGF cells with 1~10 μM CsA, mRNA levels of Fas, a component of death receptor assemblies, was determined using RT-PCR analysis. CsA downregulated Fas in mRNA levels in a dose-dependent manner(A). CsA decreased caspase-8 activity at 1 μM concentration CsA (B).(Results are mean±SD from 5 experiments.)

5. Fas was downregulated in CsA-treated HGF

To know whether death receptor-mediated apoptotic signal is involved in proliferation of HGF cells, the mRNA levels of Fas, a death receptor assembly, were determined using RT-PCR. Expression of Fas was downregulated in response to 1~10 μM CsA for 8h in a dose-dependent manner(Figure 8). This result suggests that death receptor-mediated apoptotic signal may play a crucial role in CsA-induced proliferation of the HGF cells.

Ⅳ. Discussion

Cyclosporin A(CsA), a cyclic polypeptide composed of 11 aminoacids, has been used immunosuppressive drug to prevent organ transplant rejection and to treat autoimmune diseases. One of prominent side effects in CsA therapy is gingival overgrowth, which is basically characterized by accumulation of extracellular matrix within the gingival connective tissues and an increment of HGF proliferation. However, there has been controversy whether CsA-induced gingival overgrowth represents a true hyperplasia or not. Some previous reports demon-

strated that CsA has a stimulatory effect on human gingival fibroblast[6,8]. In contrast, other previous reports showed that CsA has no effect or a inhibitory effect on proliferation in gingival fibroblast[10,11]. These variant effects of CsA may be contributed to subpopulation of human gingival fibroblast which have different characteristics in response to CsA[21]. Moreover, previous reports have showed that CsA has different effects on cell proliferation according to cell type. CsA interferes with T cell proliferation whereas it promotes hair epithelial cell proliferation[22,23].

In the present study, CsA promoted proliferation of HGF over 0.1-10 μM, in a dose- and time-dependent manner, confirming that CsA has a stimulating effect on HGF, even if CsA induced HGF cell death at high concentration above 50 μM CsA. Concentration(0.1-10 μM) of CsA used in the present study is similar to the plasma concentration(100-200 μg/ml) of patients undergoing CsA treatment. A recent study reported that transforming growth factor-β1 is involved in enhanced proliferation by CsA in HGF[12]. However, there is another possibility that apoptotic signals and its regulatory molecules may play a role in CsA-induced proliferation in HGF since cell survival rate depends on the balance between cell proliferation and cell death. Indeed, overexpression or upregulation of Bcl-2, an antiapoptotic molecule which release cytochrome *c* from mitochondria into cytosol, promotes myocyte or vascular endothelial cell proliferation[24,25]. Besides, Bcl-2 promotes regeneration of severed axon in mammalian CNS[15]. These previous reports support the possibility that apoptotic signals and its regulatory molecules may be involved in CsA-induced HGF proliferation. Up to date, however, there are no reports which examine the role of apoptotic signals and its regulatory molecules in CsA-induced proliferation of HGF. Interestingly, present study showed that Bcl-2, an antiapoptic molecule which inhibits cytochrome c release from mitochondria into cytosol, was upregulated, whereas Bax and Bid, apoptotic molecules which oppositely, to Bcl-2, were down regulated. From the present results, it is suggested that Bcl-2 family may play a pivotal role in CsA-induced proliferation of HGF. Recently, some previous reports demonstrated that Bcl-2 family controls cell proliferation through directly regulating the cell cycle, supporting the possibility that Bcl-2 family may play a role in CsA-induced proliferation in HGF[26-28]. Beside, another previous report showed that Bcl-2 regulates cytosolic reactive oxygen species(ROS) level, a potent apoptotic stimulator which stimulates, resulting into stimulating cell proliferation[29]. In the present study, cytosolic ROS levels were significantly reduced in CsA-treated HGF. From the previous reports and the present results, it is suggested the possibility that CsA stimulates cell proliferation through reducing cytosolic ROS level by regulating Bcl-2 family in HGF, even if its underlying mechanisms are still unknown. However, the mechanisms regulating expression of Bcl-2 family by CsA were not examined in the present study, of which further studies will be needed.

On the other hand, it has been well known that two distinct pathways execute apoptotsis regulated by Bcl-2 family, ultimately classifying into mitochondriadependent pathway(intrinsic pathway) and death receptor-dependent(extrinsic pathway)[30]. Mitochondria-dependent apoptotic pathway is initiated when cytochrome *c* is released through voltage dependent anion channel(VDAC) from the impaired mitochondria into cytosol, subsequently forming complexes with Apaf-1 in the presence of dATP of its analogies that recruits and activates caspase-9 which activates caspase -3 [31,32]. Death receptor-dependent apoptotic pathway is driven from caspase-8 activated through Fas or TNF-α receptor system. Caspase-8 then activates caspase-3 through direct proteolytic action or through activation mitochondria-dependent pathway by cleaving Bid, a member of the mitochondria-damaging proapotptotic Bcl-2 family [33,34]. However, none of experiments have showed the evidence which apoptotic signals are involved in CsA-induced cell proliferation in HGF. In the present study, the activity of caspase-3, a key and common caspase, in both mitochondria- and death receptor-mediated apoptosis, was diminished in CsA-treated HGF, indicating that apoptotic signals may involved in CsA-induced cell proliferation of HGF. In the present study, both caspase-9 activity and cytosolic cytochrome *c* level were almost reduced in CsA-treated HGF, even if cytosolic cytochrome *c* was slightly elevated at low concentration of CsA. These results suggest the possibility that mitochondria-meidated apoptotic signals may involved in CsA-induced cell proliferation in HGF. Many previous reports have showed that CsA is a specific VDAC permeability transition pore (PTP) blocker which inhibiting cytochrome *c* release from mitochondria into cytosol and Bcl-2 family directly regulates VDAC-PTP opening[35-38]. From the previous reports, it is assumed that CsA reduces cytosolic cytochreme *c* through directly blocking VDAC-PTP opening or regulating Bcl-2 expression in HGF. In addition, surprisingly, expression of VDAC 3 and VDAC 1 among the VDAC family were downregulated in CsA-treated HGF. The present results show the possibility that CsA inhibits cytochrome *c* release from mitochrondira into cytosol not only through directly blocking VDAC-PTP, but also by regulating VDAC expression.

In addition, expression of Fas, a death receptor assembly, was downregulated in CsA-treated HGF. Moreover, expression of Bid, which is known to be activated by caspase-8 was downregulated in CsA-treated HGF. Taken together, it is speculated that the death receptor-mediated apoptotic signals as well as mitochondria-mediated apoptotic signals may be involved in CsA-induced cell proliferation, of which their underlying mechanisms will be further studied to elucidated.

In summary, the present results suggest that Bcl-2 family and apoptotic signal may play a crucial role in CsA-induced cell proliferation in HGF.

V. References

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-국문초록-

사람 치은 섬유모세포에서 Cyclosporin-A 유도 세포증식에 대한 항세포고사 기전

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Cyclosproin A(CsA)는 세포 이식거부방지를 위한 면역 억제제 및 자가 면역질환 치료제로 널리 사용되어 왔다. CsA는 배양된 사람 치은섬유아세포를 증식시킴이 알려져 있지만 CsA에 의한 세포증식기전에 대한 세포사멸기전 및 Bcl-2의 역할은 연구되어 있지 않다.

이번 연구는 사람 섬유아세포에서 CsA에 의한 세포증식기전에 세포고사기전 및 Bcl-2 family가 관여하는지 밝히는 데에 목적이 있다. 세포 생장력은 MTT 방법으로 측정하였다. Bcl-2 family와 Fas 발현 정도는 RT-PCR 방법이나 western blot으로 확인하였다. Caspase-3 및 -9의 활성은 ELISER reader로, reactive oxygen species(ROS)는 fluorescence spectrometer에 의해 측정되었다. 미토콘드리아에서 세포질로 분비된 cytochrome *c*는 Western blot으로 조사하였다.

CsA는 0.1~10 μM에서 사람 섬유아세포의 생존률을 시간과 농도 의존적으로 증가시켰으며, 50 μM CsA에서는 오히려 세포가 죽었다. 또한, CsA 처리로 미토콘드리아에서 세포질로 유리되는 cytochrome *c* 양과 VDAC 1 및 3 발현량이 감소되었고, caspase-9과 caspase-3의 활성도도 감소되었다. 한편, CsA 처리한 섬유아세포에서 death receptor 구성요소인 Fas 발현이 감소되었다. Bcl-2 family에 대한 RT-PCR, western blot 분석결과, 세포고사를 억제하는 Bcl-2 발현은 증가되었으나 세포고사를 자극하는 Bax와 Bid의 발현은 감소되었다.

이러한 결과들은 사람 섬유아세포에서 CsA유도 세포증식에 Bcl-2 family와 ROS가 매개하는 미토콘드리아 의존 및 death receptor 의존 세포고사기전이 관여함을 시사하였다.

Key words : 치은 섬유모세포, Cyclosporin-A, caspase, Bax, Bid, Bcl-2