β-Hydroxyisovalerylshikonin induces apoptosis and G0/G1 cell-cycle arrest of endometriotic stromal cells: a preliminary in vitro study

M.Nishida, K.Nasu1, T.Ueda, A.Yuge, N.Takai and H.Narahara

Department of Obstetrics and Gynecology, Faculty of Medicine, Oita University, Oita, Japan

1To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Faculty of Medicine, Oita University, Idaigaoka 1-1, Hasama-machi, Yufu-shi, Oita 879–5593, Japan. E-mail: nasu@med.oita-u.ac.jp

BACKGROUND: Most of the current medical treatments for endometriosis aim to down-regulate the estrogen activity. However, a high recurrence rate after medical treatments has been the most significant problem. β-Hydroxyisovalerylshikonin (β-HIVS) is an ATP non-competitive inhibitor of protein-tyrosine kinases and is considered an apoptosis-inducing agent. The aim of this study is to evaluate the effects of β-HIVS on the proliferation, cell cycle and apoptosis of endometriotic stromal cells. METHODS: We investigated the effects of β-HIVS on cultured ovarian endometriotic cyst stromal cells (ECSC) by a modified methylthiazoletetrazolium (MTT) assay, a 5-bromo-2'-deoxyuridine (BrdU) incorporation assay and internucleosomal DNA fragmentation assays. The effect of β-HIVS on the cell cycle of ECSC was determined by flow cytometry. The expression of apoptosis-related molecules was examined in ECSC using western blot analysis. RESULTS: β-HIVS significantly inhibited the proliferation and DNA synthesis of ECSC and induced apoptosis and G0/G1 phase cell-cycle arrest of these cells. Down-regulation of the B-cell lymphoma/leukaemia-2 (Bcl-2) expression with the activation of caspase-3, caspase-8 and caspase-9 was observed in ECSC after β-HIVS treatment. CONCLUSIONS: These results suggest that β-HIVS induces apoptosis of ECSC by suppressing anti-apoptotic proteins. Although our present findings are preliminary, β-HIVS could potentially be a therapeutic agent for the treatment of endometriosis.

Key words: apoptosis/Bcl-2/cell cycle/endometriosis/β-hydroxyisovalerylshikonin

Introduction

Endometriosis, a common, benign, estrogen-dependent disease affecting 3–10% of women of reproductive age, is characterized by the ectopic growth of endometrial tissue (Olive and Schwartz, 1993). It is found primarily in the peritoneum, ovary and rectovaginal septum. Women suffering from endometriosis may present with chronic pelvic pain, dysmenorrhea, dyspareunia and subfertility. The prevalence of endometriosis in women with pelvic pain and/or subfertility is estimated to be between 20 and 90%; thus, it is one of the most frequently encountered benign gynaecological problems (Gazvani and Templeton, 2002).

Various therapies have been used for treating endometriosis, including surgical and medical strategies. Surgical intervention is the treatment of choice, because it has been shown that the ablation of the endometriotic lesions increases the pregnancy rate in infertile women (Marcoux et al., 1997) and reduces pelvic pain in symptomatic patients (Sutton et al., 1997). Historically, medical therapies have included contraceptive steroids, progestogens and agonists of GnRH, as well as androgens and non-steroidal anti-inflammatory agents (Lessey, 2000; Practice Committee of the American Society for Reproductive Medicine, 2004). Current medical treatment aims to inhibit the growth of endometriotic implants by suppressing ovarian steroids and inducing a hypoestrogenic state (Lessey, 2000; Practice Committee of the American Society for Reproductive Medicine, 2004). Of these medical agents, GnRH agonists have gained predominance in the medical treatment of endometriosis. GnRH agonists suppress the release of FSH and LH from the pituitary gland and inhibit ovarian steroidogenesis, resulting in a hypoestrogenic state that is suitable for the remission of the endometriotic lesions (Bergqvist et al., 1998). However, current treatments for endometriosis that aim to lower circulating estradiol (E2) concentrations can be used only for a limited time owing to unacceptable side effects. In addition, high recurrence rates after medical treatments are the most significant problem (Bergqvist, 2000). Therefore, novel therapeutic strategies are necessary for the improvement of clinical management in patients with endometriosis.

Endometriosis is believed to be the result of implantation following retrograde shedding of endometrium during menstruation. Endometrial fragments that develop into endometriotic...
lesions have the capacity to adhere, attach and implant ectopically (Koks et al., 1999; Maas et al., 2001). It has been suggested that, in healthy women, endometrial cells expelled during menstruation do not survive in ectopic locations because of programmed cell death, whereas decreased apoptosis may lead to the ectopic survival and implantation of these cells and the development of endometriosis (Gebel et al., 1998; Jones et al., 1998; Nishida et al., 2005). Both the inability of endometrial cells to transmit a ‘death’ signal and the ability of endometrial cells to avoid cell death have been associated with the increased expression of anti-apoptotic factors [e.g. B-cell lymphoma/leukaemia-2 (Bcl-2) and Bcl-XL] and the decreased expression of pre-apoptotic factors [e.g. Bax] (Jones et al., 1998; Nishida et al., 2005). These findings encouraged the authors to evaluate apoptosis-inducing agents for the treatment of endometriosis.

The naphthoquinone pigment, shikonin is the enantiomer of alkannin, which has multiple pharmacological actions including anti-bacterial, anti-fungal, anti-inflammatory, anti-thrombotic, anti-tumour, anti-gonadotrophic and anti-human immunodeficiency virus activities (Chen et al., 2002). Extracts of the roots of Lithospermum radix, which contain shikonin and various derivatives of shikonin, were used in ancient Japan for the preparation of ointments for the treatment of cuts and burns. They were also taken internally as an antidote for various poisons and as an antipyretic and anti-inflammatory agent. β-Hydroxyisovalerylshikonin (β-HIVS) (Figure 1), a representative derivative of shikonin, is an ATP non-competitive inhibitor of protein-tyrosine kinases (Hashimoto et al., 2002). In terms of its anti-tumour activities, β-HIVS has the strongest apoptosis-inducing activity among various derivatives of shikonin (Hashimoto et al., 1999). β-HIVS has been demonstrated to inhibit the growth of tumour cells, such as leukaemia (Hashimoto et al., 1999; Masuda et al., 2004; Xu et al., 2004), malignant melanoma (Hashimoto et al., 1999), renal cancer (Hashimoto et al., 2002), breast cancer (Hashimoto et al., 2002), ovarian cancer (Hashimoto et al., 2002) and lung cancer (Hashimoto et al., 2002; Masuda et al., 2004; Xu et al., 2004), by inducing the apoptosis and cell-cycle arrest of these cells.

In this study, we evaluated the effects of β-HIVS on the proliferation, cell cycle and apoptosis of cultured endometriotic cells using methods that were previously proposed for evaluating novel therapeutic agents for endometriosis (Nasu et al., 2005; Nishida et al., 2005). We also discuss the possible application of this agent for the medical treatment of endometriosis.

**Materials and methods**

**Endometriotic cyst stromal cells and normal endometrial stromal cells isolation procedure and cell culture conditions**

Endometriotic cyst stromal cells (ECSC) were obtained from premenopausal patients who had undergone salpingo-oophorectomy or cystectomy for ovarian endometriotic cysts (n = 8). Normal endometrial stromal cells (NESC) were obtained from premenopausal patients who had undergone hysterectomies for subserosal leiomyoma (n = 6). None of the patients had received any hormonal treatments before the operation. All specimens were diagnosed as being in the mid-to-late secretory phase and considered as unaffected by the presence of leiomyoma using a standard histological examination of endometrial tissues. This study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Oita University, and written informed consent was obtained from all patients.

ECSC were isolated from ovarian endometriotic tissues by enzymatic digestion as previously described (Nishida et al., 2004). Briefly, the tissues were minced in Hanks’ balanced salt solution and digested with 0.5% collagenase (Gibco-BRL, Gaithersburg, MD, USA) in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco-BRL) at 37°C for 40 min. The dispersed cells were filtered through a 70-μm nylon mesh to remove the undigested tissue pieces. The filtered fraction was separated further from the epithelial cell clumps by differential sedimentation at unit gravity as follows. The cells were resuspended in 2 ml of culture medium and layered slowly over 10 ml of the medium in a centriﬁuge tube. Sealed tubes were placed in an upright position at 37°C in 5% CO2 in air for 30 min. After sedimentation, the top 8 ml of the medium was collected. Finally, the medium containing stromal cells was ﬁltered through a 40-μm nylon mesh. Final puriﬁcation was achieved by allowing stromal cells, which attach rapidly to plates, to adhere selectively to the culture dishes for 30 min at 37°C, followed by the removal of non-adhering epithelial cells. NESC were also isolated from endometrial tissues by enzymatic digestion, as previously described (Nishida et al., 2004). Isolated ECSC and NESC were cultured in DMEM supplemented with 100 IU/ml of penicillin (Gibco-BRL), 50 mg/ml of streptomycin (Gibco-BRL) and 10% heat-inactivated fetal bovine serum (FBS) (Gibco-BRL) at 37°C in 5% CO2 in air.

ECSC and NESC in the monolayer culture after the third passage were >99% pure as analysed by immunocytochemical staining with antibodies to vimentin, CD10, cytokeratin, factor VIII and the leukocyte common antigen and were used for the following experiments. Each experiment was performed in triplicate and repeated at least four times.

**Assessment of cell proliferation and cell viability of ECSC and NESC**

Cell proliferation and the cell viability of ECSC and NESC after β-HIVS treatment were determined in 96-well plates by a modified methylthiazoletetrazolium (MTT) assay using WST-1 (Roche Diagnostics, Penzberg, Germany) according to the manufacturer’s protocols. A total of 5 × 102 cells in DMEM supplemented with 10% FBS were distributed into each well of a 96-well flat-bottomed microplate (Corning, New York, NY, USA) and incubated overnight. The medium was then removed, and the cells were incubated for 48 h with 200 μl of experimental medium containing various concentrations of β-HIVS (0.1–100 nM) (Nagara Science, Gifu, Japan). Thereafter, 20 μl of WST-1 dye was added to each well, and the cells were further incubated for 4 h. Cell proliferation was evaluated by measuring the absorbance at 540 nm. Data were calculated as the ratio of values obtained for the β-HIVS-treated cells to those for the untreated controls.
Cell proliferation of ECSC and NESC after β-HIVS treatment was also determined by 5-bromo-2′-deoxyuridine (BrdU) incorporation using cell proliferation ELISA (Roche Diagnostics). A total of $1 \times 10^6$ cells in DMEM supplemented with 10% FBS were distributed into each well of a 96-well flat-bottomed microplate and incubated overnight. The medium was then removed, and the cells were incubated for 48 h with 100 μl of experimental medium containing various concentrations of β-HIVS (0.1–100 nM). Thereafter, 10 μl of BrdU (10 mM) was added to each well and further incubated for 2 h. BrdU incorporation was then evaluated according to the manufacturer’s protocols. Cell proliferation was evaluated by measuring the absorbance at 450 nm. Data were calculated as the ratio of values obtained for the β-HIVS-treated cells to those for the untreated controls.

### Assessment of internucleosomal DNA fragmentation in ECSC

Internucleosomal DNA fragmentation in ECSC after β-HIVS treatment was evaluated by a Quick apoptotic DNA ladder detection kit (BioVision Research Products, Mountain View, CA, USA) as previously described (Nishida et al., 2005). A total of $1 \times 10^5$ cells of ECSC were plated on 100-mm culture dishes (Corning) in 10 ml of DMEM supplemented with 10% heat-inactivated FBS and cultured overnight. The supernatant was replaced with fresh culture medium containing β-HIVS (100 nM). Twenty-four hours after stimulation, the DNA was extracted from these cells according to the manufacturer’s protocols. DNA fragmentation was analysed by electrophoresis on an agarose gel (1.2%). The DNA bands were visualized by staining with ethidium bromide and photographed under UV light using a transilluminator.

### Assessment of β-HIVS-induced apoptosis in ECSC and NESC

The β-HIVS-induced apoptosis of ECSC and NESC was quantified by the direct determination of nucleosomal DNA fragmentation with a cell death detection ELISA (Roche Diagnostics) as previously described (Nishida et al., 2005). The assay used specific monoclonal antibodies directed against histones from fragmented DNA, allowing the determination of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. Briefly, $1 \times 10^5$ cells were plated on 24-well culture plates (Corning) in 1 ml of culture medium with 10% heat-inactivated FBS and cultured overnight. The supernatant was replaced with fresh culture medium containing various amounts of β-HIVS (0.1–100 nM). Twenty-four hours after stimulation, the cells were lysed according to the manufacturer’s manual, followed by centrifugation (200 $\times$ g, 5 min). The mono- and oligonucleosomes contained in the supernatants were determined using an anti-histone–biotin antibody. The concentration of nucleosomes’ antibody was determined photometrically at a wavelength of 405 nm using 2,2′-azino-di[3-ethylbenzthiazolium-sulphonate] as substrate. Data were calculated as the ratio of values obtained for the β-HIVS-treated cells to those for the untreated controls.

### Analysis of cell cycle by flow cytometry

The cell cycle was analysed by flow cytometry after 2 days of culture either with or without β-HIVS, as previously described (Nasu et al., 2005). Briefly, ECSC were cultured at <60% confluence for 2 days with or without the presence of β-HIVS (100 nM), trypsinized, washed in phosphate-buffered saline (PBS), fixed in ethanol and incubated for 30 min at 4°C in the dark with a solution of 5 μg/ml of propidium iodide, 1 mg/ml RNase (Sigma) and 0.1% Nonidet P-40 (Sigma). Flow cytometrical analysis of the cell cycle was performed immediately after staining using the CELLFit program (Becton-Dickinson, Sunnyvale, CA, USA), in which the S-phase was calculated using an RFit model.

### Assessment of the expression of apoptosis-related proteins in ECSC

The expressions of apoptosis-related proteins (Bcl-2, Bcl-XL, Bak, Fas, Fas ligand, caspase-3, caspase-8 and caspase-9) in ECSC were investigated by western blotting analysis. Subconfluent ECSC were cultured on 100-mm dishes for 24 h with or without the presence of β-HIVS (100 nM). The cells were then washed with PBS, and whole cell extracts were prepared by lysing the cells in lysis buffer (50 mM Tris–HCl, 125 mM NaCl, 0.1% Nonidet P-40, 5 mM EDTA, 50 mM NaF and 0.1% phenylmethylsulphonyl fluoride). The suspension was centrifuged at 15 000 $\times$ g for 15 min at 4°C, and the supernatant was collected. The total protein concentration was quantified using the Coomassie protein assay reagent (Pierce, Rockford, IL, USA). The whole cell protein extract was resolved with sodium dodecyl sulphate–polyacrylamide gel electrophoresis using a 10% polyacrylamide gel under reduced conditions. After the transfer to an Immobilon-P transfer membrane (Millipore, Bedford, MA, USA), the protein was stained with Ponceau S (Sigma) to verify uniform loading and transfer. The membranes were blocked with 5% skim milk (Becton-Dickinson) in Tris-buffered saline with Tween-20 (TBS−T) (50 mM Tris–HCl, 150 mM NaCl, 0.1% Tween-20, pH 7.4) (TBS-T) overnight and subsequently incubated with primary antibodies (Bcl-2, Bcl-XL, Bak, Fas and Fas ligand (BD Biosciences, San Jose, CA, USA), cleaved caspase-3, cleaved caspase-8, cleaved caspase-9 (Cell Signaling, Beverly, MA, USA) and glyceraldehyde-3-phosphatedehydrogenase (GAPDH) (Ambion, Austin, TX, USA)] at an appropriate dilution for 1 h at room temperature. The membrane was washed three times with TBS−T and incubated with the appropriate horse-radish peroxidase-conjugated secondary antibody for 1 h at room temperature. Subsequently, the membrane was washed three times with TBS−T and analysed by enhanced chemiluminescence (Amersham Pharmacia Biotech, Chicago, IL, USA).

### Statistical analysis

Data were calculated as percentages relative to the untreated controls, presented as the means ± SD and appropriately analysed by the Bonferroni/Dunn test or Mann–Whitney U-test with StatView 4.5 (Abacus Concepts, Berkeley, CA, USA). Values of $P < 0.05$ were accepted as statistically significant.

### Results

#### Effects of β-HIVS on the cell proliferation and cell viability of ECSC and NESC

The effects of β-HIVS on the cell proliferation and cell viability of ECSC and NESC were investigated by modified MTT assay. As shown in Figure 2A, the number of viable ECSC and NESC was significantly decreased by the addition of increasing amounts of β-HIVS (76.3 and 60.5% decrease at a concentration of 100 nM, respectively). β-HIVS showed a stronger inhibitory effect on the cell viability of ECSC in comparison with that of NESC (Figure 2B).

To further assess the effects of β-HIVS on the cell proliferation, the DNA synthesis of ECSC and NESC after β-HIVS treatment was evaluated by a BrdU incorporation assay. As shown in Figure 3, β-HIVS treatment resulted in a significant inhibition of the BrdU incorporation of ECSC in a dose-dependent manner (70.1% decrease at a concentration of 100 nM). Whereas β-HIVS was associated with a weak inhibitory effect on the BrdU incorporation of NESC (52.2% decrease at a concentration of 100 nM).
β-HIVS-induced apoptosis of endometriotic cells

Effects of β-HIVS on apoptosis of ECSC and NESC
The apoptotic effects of β-HIVS on ECSC and NESC were assessed by evaluating the presence of internucleosomal DNA fragmentation. β-HIVS induced the fragmentation of internucleosomal DNA in ECSC (Figures 4 and 5). A DNA ladder was detected by electrophoresis in the β-HIVS-treated ECSC, suggesting the presence of apoptotic cells (Figure 4). The apoptosis of ECSC and NESC was significantly induced by the addition of increasing amounts of β-HIVS (773.9 and 497.2% increase at a concentration of 10 nM, respectively) (Figure 5A).

Effects of β-HIVS on the cell cycle of ECSC
The effect of β-HIVS on the cell cycle of the ECSC was determined by flow cytometry. As shown in Figure 6, the culture of ECSC for 2 days in the presence of β-HIVS (100 nM) resulted in an accumulation of these cells in the G0/G1 phase of the cell cycle, with a concomitant decrease in the proportion of those in the S phase. Similar results were obtained in all repeated experiments.

Effects of β-HIVS on the expression of apoptosis-related and cell-cycle-related proteins in ECSC
To analyse the underlying mechanisms of the above findings, we evaluated the expression of apoptosis-related and cell-cycle-related proteins in ECSC. As shown in Figure 7, β-HIVS down-regulated the expression of Bcl-2 protein and up-regulated the expression of the cleaved caspase-3, cleaved caspase-8 and cleaved caspase-9 protein in ECSC. The levels of Bax, Fas, Fas ligand and Bcl-XL protein in ECSC were unchanged.

β-HIVS showed a stronger stimulatory effect on the apoptosis of ECSC than on the apoptosis of NESC (Figure 5B).

Effects of β-HIVS on the cell cycle of ECSC
The effect of β-HIVS on the cell cycle of the ECSC was determined by flow cytometry. As shown in Figure 6, the culture of ECSC for 2 days in the presence of β-HIVS (100 nM) resulted in an accumulation of these cells in the G0/G1 phase of the cell cycle, with a concomitant decrease in the proportion of those in the S phase. Similar results were obtained in all repeated experiments.

Effects of β-HIVS on the expression of apoptosis-related and cell-cycle-related proteins in ECSC
To analyse the underlying mechanisms of the above findings, we evaluated the expression of apoptosis-related and cell-cycle-related proteins in ECSC. As shown in Figure 7, β-HIVS down-regulated the expression of Bcl-2 protein and up-regulated the expression of the cleaved caspase-3, cleaved caspase-8 and cleaved caspase-9 protein in ECSC. The levels of Bax, Fas, Fas ligand and Bcl-XL protein in ECSC were unchanged.
It has been suggested that decreased apoptosis in endometriotic eutopic endometrial stromal cells (Nishida et al., 1999, 2002; Masuda et al., 2003, 2004). It has been demonstrated that β-HIVS can induce the apoptosis of these cells by the inhibition of the protein-tyrosine kinase activities of the epidermal growth factor receptor and v-Src (Hashimoto et al., 2002), suppression of the polo-like kinase 1 activity (Masuda et al., 2003) and the suppression of mitochondrial tumour necrosis factor receptor-associated protein 1 expression (Masuda et al., 2004), as well as by the activation of MAP kinases (Hashimoto et al., 1999). Interestingly, β-HIVS can inhibit the proliferation of tumour cells at low concentrations between 10 and 1000 nM (Hashimoto et al., 1999). In this study, β-HIVS significantly induced the apoptosis of ECSC at a low concentration (0.1–100 nM), which was similar to the findings in previous reports (Hashimoto et al., 1999, 2002; Masuda et al., 2003, 2004; Xu et al., 2004). In contrast, weaker effects were observed in NESC, suggesting that the effects of β-HIVS might be cell-type specific and could be weaker on the normal endometrium.

Although endometriosis is considered a benign disorder, endometriotic cells have been shown to exhibit various neoplastic potentials such as anti-apoptotic, angiogenic, invasive and metastatic abilities (Gaetje et al., 1995; Jimbo et al., 1997; Nishida et al., 2005). Apoptosis-inducing agents specific to tumour cells might be expected to be ideal anti-tumour drugs, because apoptotic cell death does not induce an inflammatory response. Chemotherapeutic agents such as cisplatin (Kaufmann, 1989), paclitaxel (Bhalla et al., 1993), camptothecin (Kaufmann, 1989), VP16 (Kaufmann, 1989) and etoposide (Kaufmann, 1989) have been shown to induce apoptosis in tumour and normal cells. However, most of these anticancer drugs have severe side effects and thus cannot be used in the medical treatment of benign diseases such as endometriosis. On the basis of its use at low concentration as an herbal remedy for centuries, β-HIVS may be found to have fewer side effects than the other chemotherapeutic agents. However, higher in vivo concentrations may be necessary for a therapeutic effect, and further clinical research would be necessary to determine the extent of gastrointestinal and bone marrow toxicity.

Current and standard medical treatments for endometriosis include GnRH agonists, contraceptive steroids, progestogens and androgens (Lessey, 2000; Practice Committee of the American Society for Reproductive Medicine, 2004), all of which aim to lower circulating E₂ concentrations. Of these agents, GnRH agonists appear to be the most effective, but they are expensive, and long-term treatment is not possible because of the loss of bone mineral density. Progestogens have the best clinical profile and a good cost-effectiveness balance; however, most studies have found that they are not as effective as GnRH agonists. Oral contraceptives are only effective during treatment and have a high relapse rate after the therapy is completed. Markedly high recurrence rates of up to 45% after current medical or surgical therapy have been reported (Bergqvist, 2000). Therefore, the need for novel medical treatments for endometriosis is an important clinical problem in routine practice. New findings on the genetics, the possible roles of the environment and the immune system and intrinsic abnormalities in the endometrium of affected women, as well as new findings on the secreted products of endometriotic...
lesions have provided insight into the pathogenesis of this disorder and have served as the background for new treatments (Nishida et al., 2004, 2005). We have demonstrated that endometriotic cells are resistant to cytokine-induced apoptosis (Nishida et al., 2005). The enhanced expression of anti-apoptotic molecules was considered to be involved in this phenomenon. As demonstrated in this study, β-HIVS can induce apoptosis of endometriotic cells by suppressing an anti-apoptotic protein, Bcl-2. Therefore, it is considered that the apoptosis-inducing mechanism of β-HIVS is suitable for the treatment of endometriosis.

In summary, we demonstrated that β-HIVS could induce apoptosis and the G0/G1 phase cell-cycle arrest of cultured endometriotic cells, which is a promising strategy for the treatment of endometriosis.

**Figure 6.** Cell-cycle analysis of endometriotic cyst stromal cells (ECSC) by flow cytometry. ECSC were cultured with or without β-hydroxyisovalerylshikonin (β-HIVS) (100 nM) for 48 h, harvested and stained with propidium iodine. (A) Representative result of untreated controls. (B) Representative result of β-HIVS-treated cells. (C) Distribution of cells in the G0/G1, S and G2/M phases of the cell cycle. *P < 0.001, **P < 0.0001 versus controls (Bonferroni/Dunn test).
ECSC. Down-regulation of the Bcl-2 expression with resultant up-regulation of the cleaved-caspase-3, cleaved caspase-8 and cleaved caspase-9 expression was induced by β-HIVS treatment. Although the safety of this agent in clinical use has not been established yet, these findings suggest that β-HIVS may be applicable for the medical treatment of endometriosis. Because the present study involves preliminary in vitro experiments, further evaluation of β-HIVS with endometriotic epithelial cells, endometriotic cells from other tissues and/or animal models is necessary to confirm the benefit of this agent for the treatment of endometriosis. In addition, further studies with other apoptosis-inducing agents on the cell proliferation and apoptosis of endometriotic cells may contribute to the establishment of more effective and sophisticated treatment strategies for endometriosis.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (no. 16591672 to K.N.).

References


Figure 7. Western blot analysis for the expression of apoptosis-related proteins (Bcl-2, Bcl-XL, Bax, Fas, Fas ligand, cleaved caspase-3, cleaved caspase-8 and cleaved caspase-9) and the glyceraldehyde-3-phosphatedehydrogenase (GAPDH) protein in endometriotic cyst stromal cells (ECSC). ECSC were cultured with or without β-HIVS (100 nM) for 24 h, and the total protein was extracted and subjected to western blotting. β-HIVS suppressed the expression of Bcl-2 protein and simultaneously induced the expression of the cleaved caspase-3, cleaved caspase-8 and cleaved caspase-9 protein in ECSC. The levels of Bax, Fas, Fas ligand and Bcl-XL protein in ECSC were unchanged.


Submitted on February 13, 2006; resubmitted on May 8, 2006, June 1, 2006; accepted on June 13, 2006

Downloaded from http://humrep.oxfordjournals.org/ at Oita University Library, Medical and Dental Branch on March 31, 2012