Growth inhibition and apoptosis induced in human leiomyoma cells by treatment with the PPAR gamma ligand ciglitizone

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The nuclear receptors PPARs (peroxisome proliferator-activated receptors) are transcription factors that play important roles in multiple disease conditions. The activation of PPARs by specific ligands is associated with growth suppression of several different types of human cancer, but the molecular mechanism responsible for this growth suppressive effect remains elusive. The aim of this study was to determine the distribution of PPARγ protein/mRNA expression in uterine leiomyomas and to identify the PPARγ induced signaling pathways responsible for the growth inhibition induced by treatment with ciglitizone, a synthetic ligand of PPARγ, in view of identifying targets that could possibly affect the viability and proliferation of uterine leiomyoma cells. Dose–response studies on proliferation found that uterine leiomyoma was more sensitive to inhibition by ciglitizone treatment than normal myometrium. We also found that ciglitizone significantly stimulated gene expression driven by a PPAR-responsive element in cultured leiomyoma cells and reduced the survival of leiomyoma cells relative to the control cells. The reduced survival of ciglitizone treated leiomyoma cells resulted from a mechanism that involved the Fas receptor-mediated apoptosis signaling cascade. These results suggest that uterine leiomyomas growth and differentiation might be modulated through PPARγ receptors and that PPARγ ligands may be of potential use for uterine leiomyoma treatment.

Keywords: uterine leiomyoma; PPARγ; ciglitizone; apoptosis

Introduction
Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor of the PPAR family, which also includes PPARα and PPAR β/δ (Sorensen et al., 1998; Desvergne and Wahli, 1999). PPARγ is activated after the binding of natural ligands such as polyunsaturated fatty acids and prostaglandin metabolites. It can also be activated by synthetic ligands, including several members of the thiazolidinedione family of ligands that includes rosiglitazone, pioglitazone, ciglitizone and troglitazone [listed from highest to lowest affinity (Kd 30–750 nM) for PPARγ] (Lehmann et al., 1995). Since the discovery of PPARs in 1990 (Issemann and Green, 1990), numerous functions have been attributed to these receptors. In addition to enhancing insulin sensitivity, PPARγ has been shown to regulate fat mass and cell proliferation (Debril et al., 2001) and to modulate inflammatory reactions. Many reports demonstrate that PPARγ ligands can inhibit the growth of multiple carcinoma cell lines via various mechanisms, including cell cycle arrest and induction of apoptosis (Fajas et al., 1997; Koeffler, 2003; Han and Roman, 2004; Yoshimura et al., 2003).

Uterine leiomyomas arise from smooth muscle cells of the uterine myometrium with an incidence rate as high as 70% in women of reproductive age. During the past two decades, progress has been made in the pathogenesis of this disease from clinical, genetic, epidemiological and molecular perspectives (Flake et al., 2003). In spite of this progress, the causes and regulatory mechanisms controlling the abnormal growth of uterine leiomyomas remain largely unknown.

PPARγ is an important signaling molecule in cells of mesenchymal origin. Although all three PPAR protein isoforms have been found in both normal myometrium and uterine leiomyoma either at the same relative levels (Houston et al., 2003) or at higher levels in leiomyoma (Tsibris et al., 1999), the PPAR signaling pathways active in uterine leiomyomas have not been clearly characterized. Identifying these pathways is of potential clinical relevance because growing evidence suggests that PPARγ ligands could have inhibitory effects on growth of leiomyomas and other types of tumors (Bruemmer and Law, 2003; Michalik et al., 2004). Loy et al. (2003) showed that growth of...
leiomymoma cells could be reduced with pioglitazone treatment. Houston et al. (2003) have shown that in uterine leiomyomas PPARγ activation is growth inhibitory, and attributed this inhibition to the negative cross talk between estrogen receptor (ER) and PPAR signaling pathways. Although other mechanisms underlying this inhibitory effect on growth has not been determined, some evidence suggests that the responses may be complex in that individual synthetic thiazolidenediones and may have differential effects on different PPAR isoforms and that the ligand-receptor interactions may also have cell type-specific biological effects.

In light of our recent work demonstrating that cigitizone plays a major role in inhibiting cell proliferation and increases [Ca^2+]i, through the activation of store-operated Ca^2+ channels in human uterine leiomyoma (Kim et al., 2005), it seemed possible that apoptosis could be involved in this growth suppression. If this hypothesis was confirmed, it would suggest that cigitizone inhibition of PPARγ signaling in uterine leiomyoma cells could promote apoptosis.

Materials and Methods

Primary tissue culture

Uterine leiomyomas and their adjacent normal myometrium tissues were obtained from hysterectomies that were conducted on benign diseases at the Keimyung University Dong San Medical Center, Daegu, South Korea. Written consent was obtained from patients, sample collection and handling were performed based on the approval and guidance of the Ethical Committee of Keimyung University. The stage of their menstrual cycle was established from each woman’s menstrual history and was verified by a histological examination of the endometrium. Uterine leiomyoma tissue samples representing the proliferative phase (Patients 1 and 2) and secretory phase (Patients 3 and 4) of the menstrual cycle were collected.

Fresh leiomyoma tissue samples were minced (Patients 1–4) and digested by incubation in Hanks solution for 4 h at 37°C, containing HEPES 0.0065 g/ml, Collagase 0.0015 g/ml and DNase 0.0002 g/ml, with periodic agitation. A portion of each tissue was stored at −70°C for isolation of mRNA and proteins. The dispersed myometrial and leiomyoma cells were then centrifuged and resuspended in a culture medium consisting of Dulbecco’s modified Eagle’s medium (DMEM) supplemented with F-12 nutrient mixture, 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotic mixture and grown to confluence. Zaitseva et al. (2006) have shown that long-term cell culturing reduced the phenotypic differences between paired tissues and cells isolated from the same tissues. utmost care was taken to carry out experiments with earlier cell passages. All experiments carried out in uterine leiomyoma and normal myometrium cell cultures were from the same biopsies. Cells (1.5 × 10^6 per well) were plated in six-well plates precoated with poly-t-lysine for in vitro studies. Cigitizone was purchased from Sigma (St Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO) as the vehicle. Uterine leiomyoma and normal myometrium cells were exposed to cigitizone for 48 h at 10, 30, 50 and 100 μM concentrations and control cells received DMSO alone.

Real-time PCR

Total RNA was isolated from uterine leiomyoma and normal myometrium tissue samples by TRZol (Life Technologies, Inc., Gaithersburg, MD). Five micrograms of total RNA was reverse transcribed into cDNA using M-MLV reverse transcriptase (Promega Corp., Madison, WI, USA) according to the manufacturer’s instructions, using oligo dT primers. The RT reactions were performed at 42°C for 60 min. Real-time PCR was performed with a LightCycler 2.0 Instrument and LightCycler FastStart DNA Master SYBR Green I kit (Roche, Mannheim, Germany) according to the manufacturer’s protocol. PCR amplification mixtures (20 μl) contained 100 ng template cDNA, 4 mMol MgCl2, 5 pmol primer and 2 μl LightCycler FastStart DNA Master SYBR Green I. For detection of the human PPARγ mRNA, a combination of a sense primer, GCA GGA GCA GAG CAA AGA GG, and an antisense primer, CCA GGA ATG CTT TTG GCA TAC, was used. The following LightCycler conditions were used: initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C, annealing at 56°C for 5 s, elongation at 72°C for 5 s and cooling at 40°C for 30 s. Glyceraldehyde-3-phosphatedehydrogenase (GAPDH) RNA was used as the internal control for loading normalization.

Western blot analysis

Cell extracts were prepared in lysis buffer [10 mM Tris (pH 7.4), 5 mM EDTA, 130 mM NaCl, 1% Triton X-100, serine protease inhibitor phenylmethylsulfonyl fluoride (PMSF) (10 μg/ml), leupeptin (10 μg/ml), apro tin (10 μg/ml), 5 mM phenanthroline and 28 mM benzamidine–HCl]. Protein concentrations were measured using Bio-Rad Protein Assay Reagent (Bio-Rad, Richmond, CA, USA) following the manufacturer’s suggested procedure. Aliquots of protein were separated by 8–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA). The membrane was blocked with Tris-buffered saline containing 5% skim milk and 0.2% Tween 20. After washing, the membrane was incubated with primary antibodies, including anti-human PPARγ (Santa Cruz Biotechnology, Santa Cruz, CA, USA), PARP (Santa Cruz Biotechnology) and anti-caspase-3 (Santa Cruz Biotechnology). After reaction with horse-radish peroxidase conjugated secondary antibodies (Amersham Lifescience, Buckinghamshire, UK), bands on the membranes were visualized by an enhanced chemiluminescence (ECL) system (Amersham Lifescience).

Cell proliferation assay

To assay live cells after cigitizone treatment, uterine leiomyoma and normal myometrial cells were plated in six-well plates supplemented with DMEM, 10% FBS and F-12 nutrient mixture and then exposed to cigitizone for 48 h at 10, 30, 50 and 100 μM concentrations. The cells were then trypsinized, pelleted and then stained for 10 min with 0.2% Trypan blue solution. Live (unstained) and dead (Trypan blue positive) cells were counted in a hemocytometer chamber. The data is presented as the percentage of viable cells out of total counted cells.

Cell cycle analysis

Uterine leiomyoma cells treated for 48 h with various concentrations of cigitizone (10, 30, 50 and 100 μM) or DMSO were collected after brief trypsinization, washed with PBS and fixed in ice cold 70% ethanol. Fixed cells were treated with RNase, stained with 100 μg/ml propidium iodide and analysed by FACSscan (Becton Dickinson, Franklin Lakes, NJ, USA).

In situ detection of fragmented DNA

TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling), an immunohistochemical method that allows histologic identification of apoptotic cells, was performed. Following cigitizone treatment at 50 and 100 μM uterine leiomyoma cells were harvested, washed with PBS and then sedimented onto a microscopic slide. Residual PBS was removed, and the cells were formalin fixed, processed and embedded in paraffin. At a later time they were deparaffinized, rehydrated in graded alcohol and washed in deionized water. Protein was digested using proteinase K [20 μl/μl in phosphate-buffered saline (PBS)] to reduce background before end labeling. In brief, the procedure was as follows: fragmented 3’-OH ends were labeled with digoxigenin-dUTP by using terminal deoxynucleotidyl transferase (TdT). Peroxidase-conjugated anti-digoxigenin antibody was then reacted with the sections. Apoptotic nuclei were visualized using the peroxidase-3,3’-diaminobenzidine (DAB) reaction. Lastly, the sections were counterstained with Mayer’s hematoxylin and mounted. TUNEL positive cells were counted under a light microscope (×400). Apoptotic cells were identified as those with brown staining of the nucleus or as apoptotic bodies, which are fragments of apoptotic cells. In each experiment, the negative control was to use the label-containing solution without the enzyme solution, and the positive control was sections exposed for 10 min at room temperature to DNaseI (1 mg/ml) in 40 mM Tris–HCl, 6 mM MgCl2, pH 7.5) prior to performing the TUNEL reaction. Fluorescin isothiocyanate-staining for TUNEL-positive cells for visualization of in situ apoptosis was carried out after cigitizone treatment (50 and 100 μM).
DNA laddering analysis

Cleavage of DNA into oligonucleosomal fragments, recognizable as a DNA ladder when electrophoresed on an agarose gel, is usually considered the biochemical hallmark of apoptosis. Ciglitizone treatment of uterine leiomyoma cells was similar to that described under in situ detection of fragmented DNA. Apoptosis detached cells and remaining adherent cells were pooled together for DNA extraction followed by centrifugation at 200 × g for 5 min. The pellet containing both the adherent and detached apoptotic cell fractions was treated with extraction buffer, to isolate apoptotic DNA fragments from high molecular weight chromatin [8 mM Tris–HCl (pH 8.0) 0.8 mM EDTA, 0.5 mg/ml proteinase K and 4% SDS], by incubation at 50°C for 2 h. RNase A (0.5 mg/ml) was added and further incubated at 37°C for 1 h. The mixture was extracted twice with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, vol/vol). DNA was precipitated by the addition of 0.1 volume of 3 M sodium acetate and 2 volumes of ice-cold ethanol and incubated at −80°C for at least 2 h. DNA was pelleted by centrifugation at 140 × g for 15 min at 4°C, washed with 0.2 ml ice-cold 80% ethanol, air dried, resuspended in 100 μl Tris–EDTA (pH 8.0) and quantitated by absorbance at 260 nm. The samples were then loaded onto a 2% (w/v) agarose gel and electrophoresed for 4 h at constant 50 V. DNA was visualized by UV illumination.

Reporter gene assay

Uterine leiomyoma and normal myometrium cells were plated and grown for 48 h. The Effectene Transfectant Reagent kit (Qiagen, Valencia, CA, USA) was used to transfect cells with pCMV-β-galactosidase (a gift from Dr A. Butler; University of Texas M. D. Anderson Cancer Center) and PPAR-response element (PPRE)-Luc plasmids. Cells were washed twice with 1× PBS and then treated with ciglitizone for 48 h. At that time, luminescence and β-galactosidase values were determined using the Promega Luciferase Assay System (Madison, WI, USA) and Tropix Galactolight (Bedford, MA, USA) according to the manufacturer’s instructions. Luminescence was detected using a Dynex-MLX Luminometer (Chantilly, VA, USA). Luciferase activity was normalized with β-galactosidase values to correct for variations in transfection efficiency. Cells were plated at 1 × 10^6/60-mm plate in DMEM containing 5% FBS and transfected in the following day with 2 μg of PPRE-Luc plasmid (a gift from Dr R. Evans) and 0.1 ng of Renilla luciferase plasmid (Promega) using FuGene transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN, USA) at a ratio of 1 μg DNA: 3 μl FuGene. On the following day, control cells were treated with DMSO and test cells with ciglitizone at 10 μM/L. Twentyfour hours later, cells were lysed and firefly luciferase and Renilla luciferase activities were measured in a luminometer.

Caspase-8 activity test

The assay for caspase-8 activity is based on the cleavage of a chromogenic substrate Acetyl-Asp-Glu-Val-Asp-p-Nitroanilide (Ac-IETD-pNA), coupled with the caspase-8-specific inhibitor IETD-CHO. When cleaved, free pNA can be quantified using a microplate reader at 405 nm. Control and treated uterine leiomyoma cells were harvested from flasks and lysed in cell lysis buffer (50 mMol/l Tris–HCl, pH 7.5, 0.03% NP40, 1.0 mmol/l DTT) for 10 min. Lysates were then centrifuged at 12 000 × g for 12 min, and protein concentrations of the supernatant (cytosolic extract) were estimated by using the Bio-Rad Protein Assay Reagent (Bio-Rad). Equal amounts of protein extracts were loaded onto a 96-well microplate and incubated with reaction buffer, DTT, DMSO, the appropriate caspase substrates with or without the caspase inhibitors.

Statistical evaluation

All experiments were done at least in triplicate. Data are represented as the means ± SE. Student’s t-test was performed to test statistical significance. A value of P < 0.05 was considered statistically significant.

Results

Deregulation of PPARγ in human uterine leiomyoma

The relative amount of PPARγ mRNA and protein expression was determined between uterine leiomyoma and normal myometrium tissues (each pair set of uterine leiomyoma and normal myometrium tissues were isolated from the same patient). Two sets of tissue were isolated during the proliferative phase and the other two during the secretory phase by semi-quantitative real-time RT–PCR and western blot analysis, respectively.

We found higher levels of PPARγ mRNA expression in two of the uterine leiomyoma tissue samples compared with their paired normal tissue (Fig. 1A). PPARγ expression varied in uterine leiomyoma tissues samples compared with the matched myometrium. The underlying reason for the increase in PPARγ expression from leiomyoma tissues collected during the proliferative phase can be attributed to a

Figure 1: PPARγ protein and mRNA levels are increased in human uterine leiomyoma tissue and cultured cells

(A) Total RNA was isolated from uterine leiomyoma and normal myometrial tissues (four pairs of tumor and normal tissues; i.e. each pair of samples was obtained from same patient). cDNA templates for RT were prepared from the total RNA extracted. The level of PPARγ mRNA was determined by quantitative real-time PCR. Relative PPARγ mRNA levels (normalized to GAPDH) in paired samples represented as M: uterine leiomyoma, N: normal myometrium. (B) Tissue and (C) tissue cultured cell extracts (50 μg) were resolved by SDS–PAGE and electro blotted onto PVDF membranes. The blot was incubated with antibody against PPARγ. Reactive bands were visualized with an ECL labeling and detection system. β-tubulin was used as the loading control. The data represent the mean ± SE of three independent experiments. *P < 0.05 and **P < 0.01 compared with control. Samples 1, 2 are tissues isolated from patients during the proliferative phase of the menstrual cycle, and Samples 3 and 4 are tissues isolated from patients during the secretory phase.
lot of endogenous factors. Further studies with more tissue pairs are warranted to substantiate the mechanism behind the differential expression of PPARγ. However, no significant difference in the PPARγ mRNA expression in normal myometrium collected from the proliferative and secretory phase was observed. This is supported by results from studies by Ota et al. (2006).

To characterize PPARγ protein expression levels in uterine leiomyoma tissues, total cellular lysates prepared from these tissues were used for western blot analysis (Fig. 1B). We found increased PPARγ protein expression levels in the two-uterine leiomyoma tissues samples having increased PPARγ mRNA levels (Fig. 1B). To determine whether these differences in proteins levels would persist in cells cultured from these uterine leiomyoma and normal tissues from patients. Data from one of the patients (Patient 2) belonging to the proliferative phase showed similar results by western blot analysis; the uterine leiomyoma cells contained relatively more PPARγ protein than the neighboring normal tissue (Fig. 1C).

Growth inhibition of uterine leiomyoma cells by ciglitizone

Since PPARγ signaling can stimulate proliferation, we tested whether the increased PPARγ protein levels found in uterine leiomyoma cells contribute to their proliferation. We did this by blocking PPARγ signaling with various doses of PPARγ ligand ciglitizone and the total viable cells were counted by cell viability counting procedure using trypan blue dye. The results clearly show that ciglitizone caused a dose-dependent reduction in viable uterine leiomyoma cells (Fig. 2A and B). Microscopic examination revealed significant changes in cell morphology with increasing concentrations of ciglitizone, whereas the morphology of normal myometrium cells was found to be relatively unaffected by ciglitizone treatments (Fig. 2C).

Ciglitizone increases cells in sub-G1 phase of cell cycle

To identify whether the growth inhibitory effect of ciglitizone is caused by perturbation of a cell cycle-specific related event, propidium iodide staining was done so that the DNA content of untreated and ciglitizone treated leiomyoma cells could be measured by fluorescence-activated cell sorting (FACS) analysis. DNA histograms showed that ciglitizone augments the percentage of cells in the sub-G1 population at both 50 μM/L (9.39%) and 100 μM/I (12.80%), whereas at the lower doses uterine leiomyomal cells were not significantly enriched compared to control, non-treated cells (5.08%). No other significant alterations in the percentage of other cell cycle populations were observed following drug-treatment.

Ciglitizone induces apoptosis in uterine leiomyoma cells

Since the reduced proliferation of uterine leiomyoma cells treated with ciglitizone could reflect either reduced cell division or increased apoptosis, we investigated whether ciglitizone treatment induced apoptosis cascade by performing TUNEL and DNA laddering assays. The TUNEL assay is a commonly used test for evaluating DNA integrity in situ. Cultured uterine leiomyoma cells were treated with ciglitizone for 48 h at concentrations 50 and 100 μM/l and TUNEL assay was performed. Very few or none of the cells grown in the absence of ciglitizone were apoptotic by this assay (the negative control, Fig. 4A). In contrast, micronuclei associated with apoptosis were perceptible in both the 50 and 100 μM/l ciglitizone treated cultures (Fig. 4A). The presence of distinct bands, the DNA ladder, detectable after electrophoresis of genomic DNA of cells undergoing apoptosis, results from the endonuclease activity induced during apoptosis and has become a hallmark of apoptosis. DNA extracted from uterine leiomyoma cells treated with ciglitizone was electrophoresed to detect the presence of apoptosis.

![Figure 2](https://academic.oup.com/molehr/article-abstract/13/11/829/1026314/1026314)
oligonucleosome-sized fragments. The DNA from control uterine leiomyoma cells, untreated with ciglitizone, was of high molecular weight and displayed little of the increased mobility associated with apoptosis upon electrophoresis. In contrast, the DNA from uterine leiomyoma cells treated with ciglitizone at 100 μM/l concentration exhibited a DNA ladder pattern i.e. typical of apoptotic cells (Fig. 4B). These results show that ciglitizone can induce apoptosis in uterine leiomyoma cells.

**Induction of increased PPARγ reporter activity by ciglitizone**

In order to determine whether ciglitizone regulates PPARγ-mediated transcription in this cell type, a luciferase reporter assay was performed using a PPRE-Luc reporter plasmid construct. Primary cultured uterine leiomyoma and normal myometrium cells transfected with PPRE-Luc for 24 h and treated with 10 μM/L of ciglitizone for an additional 24 h were harvested for luciferase assay. As shown by

![Figure 3: Effect of ciglitizone on the cell cycle profile](image.png)

After treatment with ciglitizone (10, 30, 50 and 100 μM/l) or DMSO for 48 h, cultured uterine leiomyoma cells were harvested, fixed, stained with PI and analysed by FACS analysis. The values represent the number of cells in different phases of the cell cycle as a percentage (%) of total cells. The data shown are the average of three independent experiments.

![Figure 4: Ciglitizone induces apoptosis and DNA laddering in uterine leiomyoma cells](image.png)

(A) The effect of PPARγ ligand ciglitizone on apoptosis was measured using TUNEL assays. Following ciglitizone treatment (50 and 100 μM/L), uterine leiomyoma cells were harvested, washed with PBS and then sedimented onto microscopic slide. Apoptotic nuclei were visualized using the peroxidase-DAB reaction. TUNEL positive cells were counted under a light microscope (Magnification ×400). Apoptotic cells were identified as those with a brown staining of the nucleus or as apoptotic bodies, which are fragments of apoptotic cells. TUNEL assays for visualization of in situ apoptosis were carried out after ciglitazone treatment (50 and 100 μM/l). The results of the TUNEL positive cells were quantified. Values represent means ± SE of experiments. **P < 0.01 and ***P < 0.001 compared with control. (B) Uterine leiomyoma cells were treated with DMSO (control) or ciglitizone (10 and 100 μM/L) for 48 h. genomic DNA was extracted and run on a 2% agarose gel. Cleavage of DNA into oligonucleosomal fragments is recognized as a DNA ladder in the agarose gel.
relative luciferase values, ciglitizone treatment enhanced the transcription of the luciferase reporter driven by a PPRE-containing promoter. Luciferase activity was normalized with β-galactosidase values to correct for variations in transfection efficiency and to eliminate the differences in DNA uptake by the two cell lines. Cigli-
tizone treatment increased PPRE-Luc reporter activity more strongly in uterine leiomyoma cells than in the normal myometrial cells (Fig. 5). This shows that ciglitizone caused transactivation of PPRE in primary human leiomyoma cells.

Ciglitizone induces apoptosis potentially via Fas-mediated apoptotic pathway

In order to identify the apoptotic mechanism induced by ciglitizone on uterine leiomyoma cells, the effect of ciglitizone on caspases, Bid, Bcl-2, Bax and Fas protein expression levels, the key players in the apoptotic cascade, were examined after 48 h of exposure. Down-regulation of Bcl-2 coupled with up regulation of Bax and Fas was observed with increasing doses of ciglitizone. Ciglitizone treatment also augmented cleavage of procaspase-8 and proBid in a dose-dependent fashion. A dose-dependent decrease in procaspase-3 level coupled with decreased PARP level was also observed (Fig. 6). These results suggest that both Fas and caspase-dependent apoptosis may be involved in the ciglitizone induced apoptotic of uterine leiomyoma cells.

**Caspase activity increases after ciglitizone treatment**

Caspase activity was measured to determine if caspase-8 could be involved in initiating the death ligand apoptotic cascade pathway that appears to be induced in ciglitizone treated uterine leiomyoma cells. As expected, uterine leiomyoma cells exposed to various concentrations of ciglitizone for 48 h did show an increase in caspase-8 activity. In comparison with control cells treated with DMSO alone, the ciglitizone treated cells had an average of 30% more caspase-8 activity, with ciglitizone at 100 μM/l showing the maximum difference (69.7%) (P < 0.05) (Fig. 7).

**Discussion**

PPARγ is receiving increasing attention in the cancer field because of its increased expression in several types of carcinomas such as prostate and urinary bladder cancers (Segawa et al., 2002; Yoshimura et al., 2003). Moreover, both endogenous and synthetic PPARγ agonists elicit notable growth inhibitory effects *in vitro* and *in vivo* (colon, breast and prostate carcinomas) and are capable of preventing metastasis (Panigrahy et al., 2002; Koeffler, 2003). Several high affinity PPARγ ligands are routinely used to investigate PPARγ-mediated effects, including the family of synthetic thiazolidinedione ligands (Forman et al., 1995; Kliewer et al., 1995). We (Jung et al., 2005) and others have demonstrated that some PPARγ ligands can inhibit the growth of carcinoma cell lines derived from a variety of sources (Guan et al., 1999; Sato et al., 2000; Shiau et al., 2005) and that the
Ciglitzone induces apoptosis in leiomyoma cells

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