Anti-proliferative effect of peroxisome proliferator-activated receptor γ agonists on human malignant melanoma cells *in vitro*

Christian Freudlsperger[a], Ingrid Moll[b], Udo Schumacher[a] and Anka Thies[a]

Malignant melanoma has a poor reputation for early spread and no curative treatment is yet available. As peroxisome proliferator-activated receptor γ (PPARγ) agonists (glitazones) have recently been shown to have growth-inhibiting effects on different cancer lineages, the aim of this study was to analyze the effects of four glitazones (rosiglitazone, ciglitazone, pioglitazone and troglitazone) on the growth of six human malignant melanoma cells *in vitro*. Proliferation of six human melanoma cell lines under glitazone treatment over a broad concentration range (0.15–300 μmol/l) was assessed by means of the XTT cell proliferation assay, and expression of PPARγ in these cell lines was analyzed using both immunohistochemical and molecular biological techniques. All four glitazones showed a significant dose-dependent anti-proliferative effect on all six cell lines starting at a concentration of 0.3 μmol/l, with ciglitazone being the most potent inhibitor of cell growth, followed by troglitazone, rosiglitazone and pioglitazone. PPARγ was predominantly localized in the cytoplasm; however, there were quantitative differences in PPARγ expression between the different cell lines as demonstrated by quantification of Western blots. As an already approved class of drugs, glitazones have been found to significantly inhibit growth of human malignant melanoma cells *in vitro* and might be a promising tool for further therapeutic studies. *Anti-Cancer Drugs* 17:325–332 © 2006 Lippincott Williams & Wilkins.

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Introduction

Malignant melanoma is the most aggressive form of skin cancer and has increased more than 6-fold in incidence over the past 50 years [1]. Metastasized malignant melanoma is highly resistant to conventional chemotherapy, with dacarbazine having the best single-agent activity with a response rate ranging from 5 to 29% and a short 4-month median response duration [2]. No multiagent chemotherapy produced significantly better outcomes than single-agent dacarbazine and no evidence of prolonged overall survival in melanoma patients under chemotherapy has been presented [3]. Immunomodulating agents like interferon-α are not the breakthrough hoped for and their use has to be weighed against a considerable associated morbidity [3]. Thus, it is imperative to investigate new therapeutic targets for the treatment of melanoma to improve this dismal prognosis. Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear receptor superfamily, and is involved in cell proliferation [4], cell differentiation [5] and apoptosis [6]. After ligand binding, PPARγ functions as a key regulator of adipocyte differentiation [7] and insulin-dependent glucose utilization [8]. Prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is the most potent naturally occurring ligand for PPARγ and the glitazones, a class of anti-diabetic, insulin-sensitizing drugs, are specific exogenous ligands for PPARγ. Recently, expression of PPARγ has been demonstrated in tumor cells from various malignancies, including breast, colon, lung, gastric, pancreatic, prostate and bladder [6,9–14], and its activation led to a significant decrease in proliferation of tumor cells. As a consequence, PPARγ has become a molecular target for potential anticancer drug development.

The aim of the present study was to investigate the influence of four synthetic PPARγ agonists, i.e. rosiglitazone, ciglitazone, pioglitazone and troglitazone, on the proliferation of six human malignant melanoma cell lines *in vitro*. To analyze whether the glitazone effect is mediated through PPARγ, PPARγ expression of the melanoma cell lines was investigated using immunohistochemistry and Western blotting. To further explore glitazone function, the potential induction of apoptosis in the glitazone-treated cells was also assessed.

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Materials and methods

PPARγ agonists

The synthetic PPARγ agonists rosiglitazone, pioglitazone, ciglitazone and troglitazone were used in this study. Rosiglitazone was kindly provided by GlaxoSmithKline (Munich, Germany) and pioglitazone by Takeda Chemical Industries (Osaka, Japan), and ciglitazone was purchased from Calbiochem (Schwalbach, Germany). Troglitazone was kindly provided by M.Schachner (ZMNH, Hamburg, Germany). All glitazones were dissolved in 100% ethanol and further diluted with 10% ethanol in eight different concentrations ranging from 0.5 μmol/l to 1 mmol/l.

Cell culture and cell proliferation assays

Six different human cutaneous melanoma cell lines were used. The cell lines UISO-Mel6, established from a primary malignant melanoma [15], MV3, established from a metastatic melanoma lymph node [16] and MeWo, also derived from a metastatic melanoma lymph node [17], were kindly provided by I. Moll (Klinik für Dermatologie und Venerologie, Kopf- und Hautzentrum, Universitäts-Klinikum Hamburg-Eppendorf, Germany). The cell line G361, established from a primary malignant melanoma, was obtained from the ATCC (Manassas, Virginia, USA; no. CRL-1424). The cell lines Lox and FemX-1, both established from a metastatic lymph node [18,19], were kindly provided by Ø. Fodstad (Department of Tumor Biology, University Hospital Oslo, Norway). Cell lines were grown as monolayers in culture flasks (Nunc, Roskilde, Denmark) in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 2 mmol/l L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (all from Gibco/Invitrogen, Carlsbad, California, USA) in a 37°C humidified 5% CO_2 atmosphere. The medium was changed every 3 days and cells were separated via trypsinization using trypsin/EDTA (Gibco/Invitrogen) when they reached subconfluence. For the cell proliferation studies, cells were seeded at concentrations of 14 000 (UISO-Mel6, MV3, FemX-1), 15 000 (MeWo) or 16 000 cells/ml (Lox, G361), respectively, into 96-well culture plates (Greiner, Frickenhausen, Germany) and allowed to attach for 48 h. Cells were then treated with eight different concentrations ranging from 0.15 to 300 μmol/l per well of rosiglitazone, ciglitazone, pioglitazone or troglitazone. The final ethanol concentration was 3% per well. To exclude an influence of the solvent ethanol on the cell proliferation, the control cells were treated with 3% ethanol per well without glitazones. Cells were incubated for 48 h and cell proliferation was measured using an XTT-based assay (Roche, Mannheim, Germany). An aliquot of 50 μl of XTT solution was added for 5 h, and absorbance was measured at a test wavelength of 450 nm and a reference wavelength of 630 nm in a Dynatech MR 3.13 MicroELISA reader (Dynex Technologies, Ashford, UK). Each concentration for each test substance was tested in quadruplicate and each experiment was repeated independently 3 times.

Statistical analysis

Each concentration of each glitazone was tested in quadruplicate and in three independent experiments, resulting in 12 data points for each concentration. The effect of the glitazones on the cell proliferation of the six cell lines was studied as a dose–response experiment. Absorbance of untreated controls was taken as 100% survival and absorbance of treated cells was taken as percentage survival of the control. To ascertain statistical differences between the control cells and the treated cells, a Friedman test followed by Dunn's post-test was performed. $P < 0.05$ was considered statistically significant. To ascertain statistical differences between the total effect of the four glitazones, the areas under the curve of all six cell lines affected by one glitazone were summed and compared using a one-way ANOVA test followed by the Tukey test. All analyses were performed using GraphPad Prism version 4 (Intuitive Software for Science, San Diego, California, USA). All graphs show mean and SD.

Immunohistochemistry

For immunohistochemical analyses, human processed lipoaspirate cells (PLA), expressing PPARγ to a great extent [20], were taken as the appropriate positive control. The PLA cells were kindly provided by T. Jansen (Klinik Pöseldorf, Hamburg, Germany). Melanoma cells and PLA cells were placed on Falcon culture slides (Becton Dickinson, Franklin Lakes, New Jersey, USA) at a concentration of 1×10^5 cells/ml and cultured for 48 h. Cells were fixed in 4% paraformaldehyde for 30 min at room temperature followed by treatment with methanol and 0.1% Triton X-100 to achieve cell membrane and nuclear permeability. Non-specific binding was blocked by an incubation with normal swine serum (1 : 10; Dako, Glostrup, Denmark) for 30 min. Cells were incubated with a 1 : 50 diluted rabbit anti-human anti-PPARγ polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, California, USA) overnight at 4°C. Biotinylated 1 : 200 diluted swine anti-rabbit antibody (Dako) was incubated for 30 min at room temperature. Cells were incubated for 30 min with alkaline phosphatase-labeled streptavidin (Vectastain ABC-AP; Vector, Burlingame, California, USA). Enzyme reactivity of the alkaline phosphate complex was visualized using naphtol-AS-biphosphate and hexazotized new fuchsin was used as chromogen (all from Sigma-Aldrich, St Louis, Missouri, USA). Negative controls were treated the same way, but replacing the primary antibody with PBS. Slides were covered with Crystal Mount and Clarion (Biomedia, Foster City, California, USA), and were examined and photographed under a Zeiss Axioplan photomicroscope.

Extraction of cytoplasmic and nuclear proteins

For the cytoplasmic and nuclear protein extraction, a modified protocol was used as originally described elsewhere [21]. PLA cells served as appropriate positive control. Cells were cultured as described above and

allowed to reach 90% confluence. Cells were then washed twice in ice-cold PBS and accumulated in 1 ml PBS using a cell scraper (Greiner Bio-One, Frickenhausen, Germany). Samples were centrifuged for 30 s at 10 000 *g* and cell pellets were resuspended in 50 µl of low salt buffer (20 mmol/l HEPES, pH 7.9, 10 mmol/l KCl, 0.1 mmol/l $NaVO_4$, 1 mmol/l EDTA, 1 mmol/l EGTA, 0.2% NP-40, 10% glycerol) supplemented with 1% Protease Inhibitor Cocktail Set I (Calbiochem, San Diego, California, USA). After 10 min of incubation on ice, the samples were centrifuged at 13 000 *g* for 2 min (4°C) and the supernatants were collected as the cytoplasmic fraction. Nuclei were resuspended in high salt buffer (20 mmol/l HEPES, pH 7.9, 420 mmol/l NaCl, 10 mmol/l KCl, 0.1 mmol/l $NaVO_4$, 1 mmol/l EDTA, 1 mmol/l EGTA, 20% glycerol) supplemented with 1% Protease Inhibitor Cocktail Set I (Calbiochem) and nuclear proteins were extracted by shaking on ice for 30 min. Samples were then centrifuged at 13 000 *g* for 10 min (4°C) and the supernatants were taken as the nuclear fraction.

Western blot analysis

Protein concentrations were measured using the Bradford method [22]. Aliquots of 40 µg of protein per lane were boiled in sample buffer (0.5 mol/l Tris–HCl, pH 6.8, glycerol, 10% SDS, 0.5% bromphenol blue, mercaptoethanol) for 4 min at 95°C. Proteins were separated using 13% SDS–PAGE (Bio-Rad, Hercules, California, USA). After electrophoresis, the separated proteins were electrotransferred onto a nitrocellulose membrane (Hybond-ECL; Amersham Biosciences, Freiberg, Germany) using a Mini Trans-Blot (Bio-Rad). After blocking with 4% milk powder in PBS containing 0.5% Tween (Sigma) for 30 min, membranes were incubated with a 1 : 200 diluted rabbit anti-human anti-PPARγ polyclonal antibody (Santa Cruz Biotechnology) overnight at 4°C. The membranes were then washed with PBS containing 0.05% Tween (Sigma) and incubated with an 1 : 200 diluted polyclonal swine anti-rabbit antibody (DakoCytomation, Glostrup, Denmark) conjugated with horseradish peroxidase for 90 min at room temperature. The immune complexes were visualized using Hybond ECL (Amersham Biosciences) and X-ray films (Fuji, Tokyo, Japan) were exposed to the membranes for 1–3 min.

Quantitative analyses of the cytoplasmic PPARγ protein

Since the cytoplasmic PPARγ protein content was higher in proportion to the nuclear protein content, only the cytoplasmic proteins were used for the quantification analyses. Differences of the PPARγ protein content between the six cell lines were investigated using the quantification software Quantity One (Bio-Rad). The volume calculated by the software provides a possibility to compare the relative protein content between the six cell lines.

Detection of apoptosis

To test the possibility that apoptosis was induced by the glitazones, MV3 cells (5×10^5/ml) were grown on Falcon culture slides (Becton Dickinson) for 48 h. Three different concentrations of rosiglitazone (0.001, 0.01 and 0.1 mmol/l) were added, and cells were incubated with each concentration for 6, 12 and 24 h, respectively. After incubation, cells were fixed in 4% paraformaldehyde for 30 min at room temperature, and treated with methanol and Triton X-100 as described above. Cells were stained with 4′,6-diamidino-2-phenylindole dihydrochloride (Sigma-Aldrich, Schnelldorf, Germany). Finally, slides were dehydrated and mounted with Dako fluorescence-mounting medium (Dako), and apoptotic cells were counted under a fluorescence microscope at a wavelength of 350 nm. Furthermore, apoptosis was measured using a cellular DNA fragmentation ELISA kit (Roche, Mannheim, Germany). Exponentially growing MV3 cells were diluted with culture medium to a cell concentration of 5×10^5 cells/ml and 500 µl cell suspension was transferred into Eppendorf tubes. Then, 500 µl culture medium with two different concentrations of rosiglitazone (0.01 or 0.1 mmol/l) was added and cells were incubated for 4 h. As a positive control, 500 µl culture medium was added containing different concentrations of bortezomib (0.1 or 0.01 mg/ml) due to the fact that the anti-tumorigenic effect of bortezomib is mediated through increased apoptosis [23]. The cellular assay and the ELISA procedure were performed according to the manufacturer's manual.

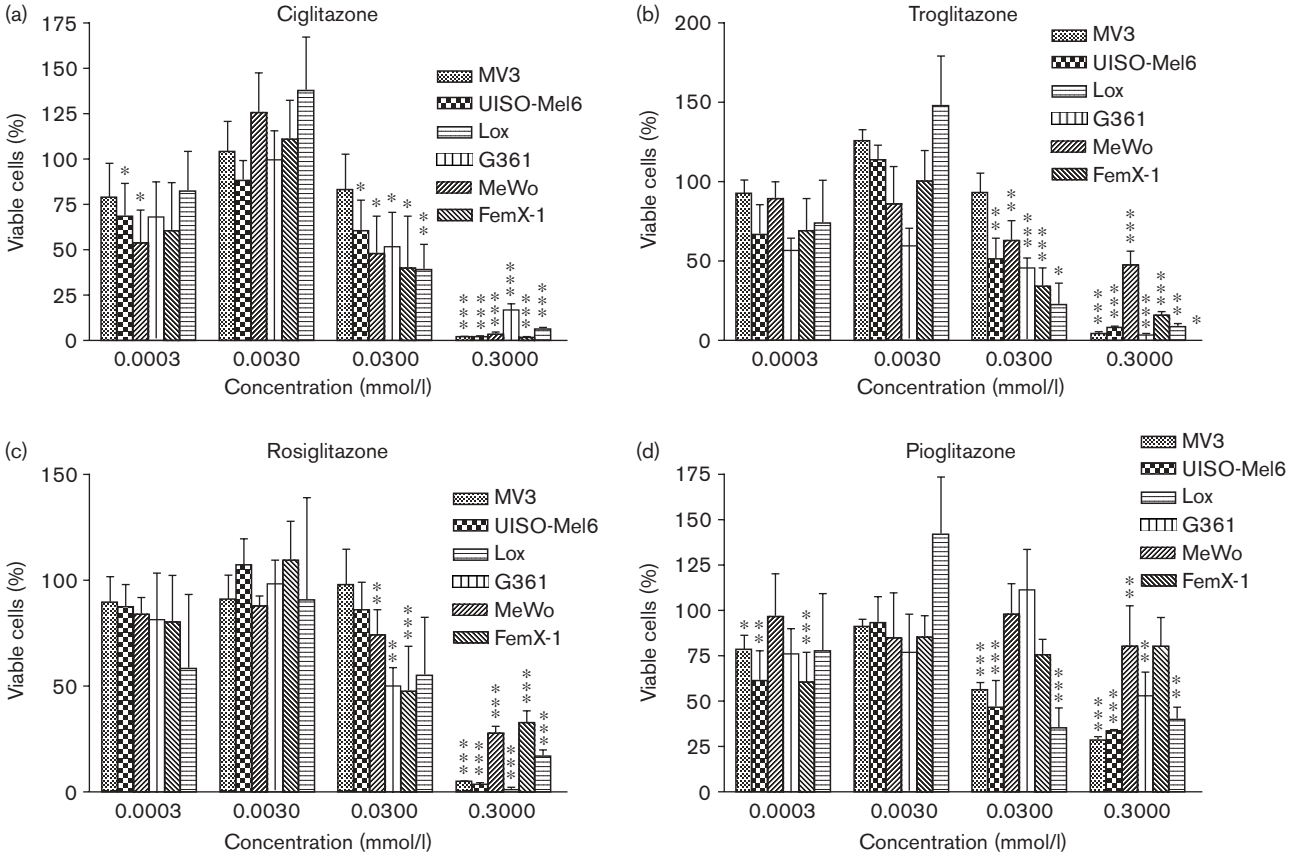
Results

Cell proliferation assays

The results of the cell proliferation assay for the four glitazones are summarized in Fig. 1(a–d). All four glitazones inhibited cell proliferation of all six human melanoma cell lines in a dose-dependent manner. In general, ciglitazone had the strongest cytotoxic effect of the four glitazones and had a comparable effect on all six cell lines (Table 1). At a concentration of 0.3 µmol/l all six cell lines showed a slight decrease in cell growth; however, inhibition was statistically significant in UISO-Mel6 and MeWo only (both $P < 0.05$). A concentration of 3 µmol/l caused a slight, but not significant, increase in cell growth in all cell lines. A concentration of 30 µmol/l ciglitazone significantly reduced the viable cells of UISO-Mel6 to 60% ($P < 0.05$), of Lox to 39% ($P < 0.01$), of G361 to 51% ($P < 0.05$), of MeWo to 48% ($P < 0.05$) and of FemX-1 to 40% of the control ($P < 0.05$). At 300 µmol/l, viable cells of all six cell lines were significantly reduced, MV3 to 2%, UISO-Mel6 to 2%, Lox to 6%, G361 to 17%, MeWo to 3% and FemX-1 to 2% of the control (all $P < 0.001$).

The second best cytotoxic effect was achieved by troglitazone, followed by rosiglitazone and pioglitazone.

Fig. 1



Effect of various concentrations of (a) ciglitazone, (b) troglitazone, (c) rosiglitazone and (d) pioglitazone on the cell growth of six human melanoma cell lines. Values indicate percentage proliferation of the respective controls. Bars and error bars represent mean and SD of three independent experiments performed in quadruplicate. $*P<0.05$, $**P<0.01$, $***P<0.001$.

Table 1 Cytotoxic effect of four glitazones at three different concentrations (0.3, 30 and 300 μmol/l) on six melanoma cell lines by means of the viable cells in terms of the percentage of untreated control cells

Drug/concentration (μmol/l)	MV3	UISO-Mel6	Lox	G361	MeWo	FemX-1
Rosiglitazone						
0.3	NS	NS	NS	NS	NS	NS
30	NS	NS	NS	51[b]	75[b]	48[a]
300	6[c]	4[c]	18[c]	2[c]	29[c]	34[c]
Ciglitazone						
0.3	NS	68[a]	NS	NS	54[a]	NS
30	NS	60[a]	39[b]	51[a]	48[a]	40[a]
300	2[c]	2[c]	6[c]	17[c]	3[c]	2[c]
Pioglitazone						
0.3	79[a]	62[b]	NS	NS	NS	61[c]
30	57[c]	47[c]	36[c]	NS	NS	NS
300	29[c]	34[c]	40[b]	54[b]	81[b]	NS
Troglitazone						
0.3	NS	NS	NS	NS	NS	NS
30	NS	51[b]	23[a]	46[c]	63[b]	34[c]
300	5[c]	8[c]	9[c]	4[c]	48[c]	16[c]

[a]$P<0.05$.
[b]$P<0.01$.
[c]$P<0.001$.

Their respective cytotoxic effects on the six cell lines were comparable to that of ciglitazone (Fig. 1b–d).

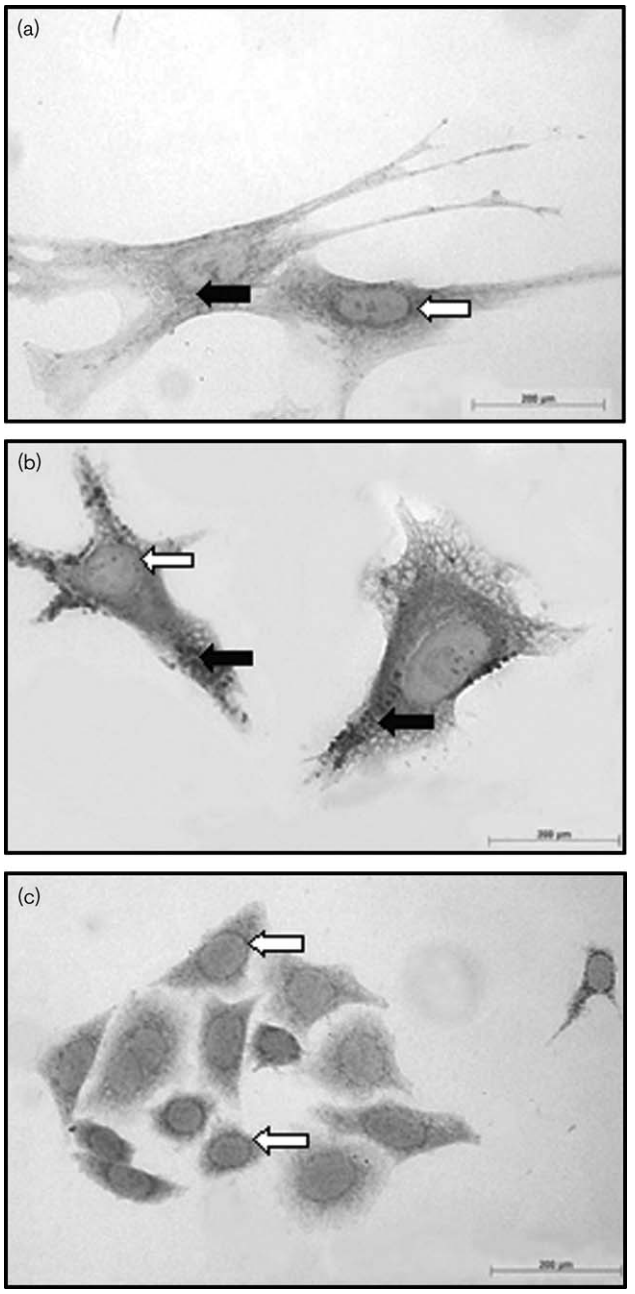
Immunohistochemical analyses

The PPARγ protein was detected in all six cell lines and in the positive control as shown in Fig. 2(a–c). The staining results were consistent in all cases, with PPARγ staining being generally confined to the cytoplasm with perinuclear accentuation, which is consistent with results seen in melanocytes [24]. Furthermore, PPARγ immunoreactivity was noticed in the nucleus.

Western blot analysis

For the Western blot analysis of the PPARγ protein, the cytoplasmic and the nuclear proteins were investigated separately. The Western blot analysis of the cytoplasmic proteins (Fig. 3) showed the presence of a single band of approximately 50 kDa for all six cell lines and the positive control, being in accordance with the expected band of

Fig. 2



Immunohistochemical detection of PPARγ in human malignant melanoma cells: (a) human PLA cells as positive control, (b) FemX-1 melanoma cells and (c) Lox melanoma cells. In all three cell lines, PPARγ staining was mostly confined to the cytoplasm (black arrows) with perinuclear accentuation (white arrows).

the PPARγ protein [12]. In the Western blot analyses of the nuclear proteins, the 50-kDa band was generally less pronounced.

Quantification of the cytoplasmic PPARγ protein

The quantification of the cytoplasmic PPARγ protein showed differences between the protein content of the six cell lines. The cell line MV3 contained the highest amount of cytoplasmic PPARγ protein, followed by Lox, MeWo, G361, FemX-1 and UISO-Mel6. To investigate which cell had the highest susceptibility towards the glitazones, the areas under the curve of cell growth inhibition of all four glitazones, affecting the respective cell line at concentrations from 15 to 300 μmol/l, were summed. Lox was the most sensitive to the glitazones, followed by UISO-Mel6, FemX-1, G361, MV3 and MeWo. Figure 4 shows the sensitivity of the cell lines in relation to the PPARγ protein content, indicating that there is no correlation between the PPARγ protein content and the sensitivity to the glitazones.

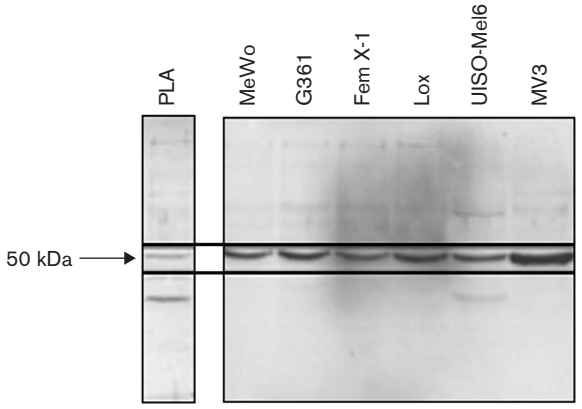
Detection of apoptosis

Cells treated with rosiglitazone were visualized under fluorescence microscopy at 350 nm for typical morphological changes indicating apoptotic cell death, e.g. chromatin condensation or nuclear fragmentation [25]. Induction of apoptosis by rosiglitazone was not noted irrespective of the concentration or duration of incubation. The results of the cellular DNA fragmentation ELISA kit (Roche) affirmed this observation (Fig. 5). Treatment with rosiglitazone could not raise the number of apoptotic cells, whereas bortezomib, as a positive control, induced apoptosis in a dose-dependent manner.

Discussion

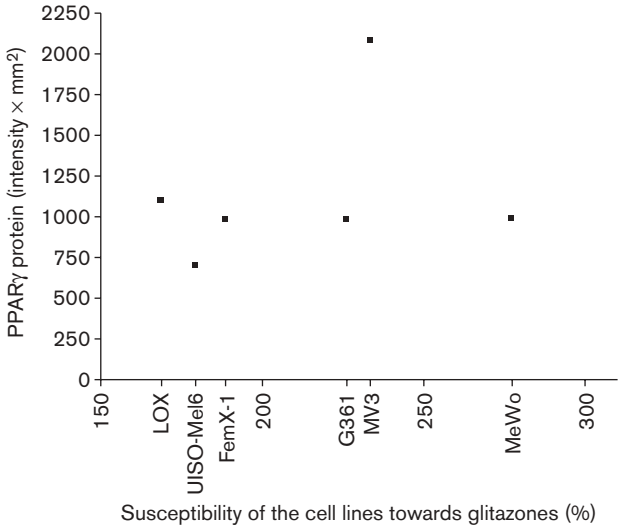
The rapid increase in incidence of malignant melanoma has not been accompanied by better therapeutic options [2,26]. Searching for alternative treatment strategies, the principal aim of this study was to investigate the in-vitro effect of four glitazones, i.e. rosiglitazone, ciglitazone, pioglitazone and troglitazone, on the cell proliferation of six different human malignant melanoma cell lines. All four glitazones had a dose-dependent anti-proliferative effect on the growth of all six melanoma cell lines *in vitro*. In general, ciglitazone had the strongest anti-proliferative effect, followed by troglitazone, rosiglitazone and pioglitazone. The concentrations of the glitazones that significantly inhibited proliferation of most of the cell lines (30 μmol/l) were comparable to those of similar growth-inhibitory potential observed by others [27]. However, Placha *et al.* reported a significant inhibitory effect on melanoma cells of ciglitazone, an ancestor of the actual glitazones, which never reached clinical use because of several side-effects [28]. As this study showed an almost equal inhibitory effect of rosiglitazone, which has found widespread acceptance for the treatment of type II diabetes, these results can be directly transferred from bench to bedside. In contrast to the growth-inhibitory effect of the glitazones at concentrations of 30 μmol/l or higher, a slight, but not significant, increase in cell proliferation of all cell lines at a glitazone concentration of 3 μmol/l was observed. This is in parallel

Fig. 3



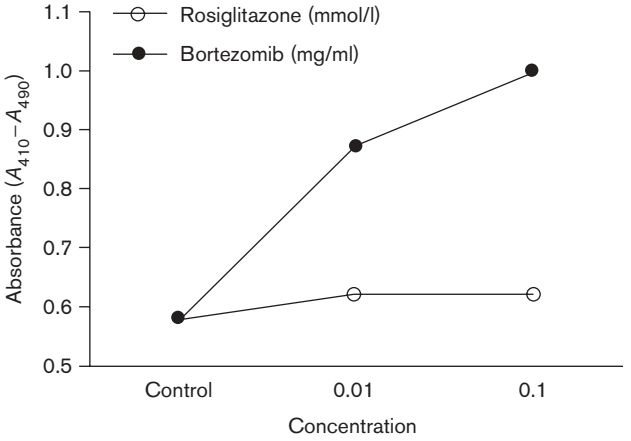
Expression of cytoplasmic PPARγ protein in all six human melanoma cell lines and in human PLA cells as determined by Western blotting. All six cell lines and the PLA cells showed the presence of a 50-kDa form.

Fig. 4



Susceptibility of the cell lines towards glitazones in comparison to their PPARγ protein content. The susceptibility represents the areas under the curve of cell growth inhibition of all four glitazones, affecting the respective cell line at concentrations from 15 to 300 μmol/l. The cell line Lox was the most sensitive, followed by UISO-Mel6, FemX-1, G361, MV3 and MeWo. The cell line MV3 contained the highest amount of cytoplasmic PPARγ protein, followed by Lox, MeWo, G361, FemX-1 and UISO-Mel6. No correlation was apparent between the susceptibility of the cell lines and their PPARγ protein content.

Fig. 5



Detection of apoptosis in MV3 cells treated with different concentrations of rosiglitazone or bortezomib by determination of cytoplasmic histone-associated DNA fragments. In contrast to bortezomib, no apoptosis was induced by rosiglitazone.

with results from Lucarelli *et al.* who reported that troglitazone, at a concentration of 5 μmol/l, promoted the survival of human osteosarcoma cells, through the activation of the phosphatidylinositol-3-kinase/Akt survival pathway [29]. However, the observed increase of proliferation in melanoma cells in the present study was far from any significance in any of the cell lines, and all experiments were tested in quadruplicate and in three independent experiments, indicating that stimulatory effects of glitazones on malignant melanoma seem to be less important. However, ongoing in-vivo studies ought to carefully address dosage investigations to eliminate any possibilities of stimulatory effects on malignant melanoma. Already conducted in-vivo studies in colon cancers showed controversial results. Sarraf *et al.* achieved a reduction of human colon carcinoma cell growth in a nude mouse model by a daily troglitazone application of 200 mg/kg body weight [10]. In contrast to this, Lefebvre *et al.* reported an induction of colon carcinoma cell growth by a daily troglitazone application of 150 mg/kg body weight in C57BL/6J-$APC^{Min/+}$ mice, an animal model susceptible to intestinal neoplasia [30], while Elstner *et al.* could inhibit the growth of human breast cancer in female triple-immunodeficient BNX mice by a daily troglitazone application of 1000 mg/kg body weight [31]. These contradictory results emphasize the results of the present study demonstrating that glitazones display their anti-tumor effect only at higher concentrations.

To further elucidate the mechanism underlying the glitazones' inhibitory effect on melanoma cell growth, the second aim of this study was to investigate whether the cytotoxic effect of the glitazones was mediated by a direct activation of PPARγ. Tsujie *et al.* demonstrated a correlation between the cytotoxic effect of glitazones on human gastrointestinal adenocarcinoma cells and the expression of PPARγ, concluding that the anti-proliferative glitazone effect is indeed mediated by an activation of PPARγ [32]. Possible mechanisms for the anti-proliferative effect of glitazones are (i) an induction of

G_1 cell cycle arrest, as observed in colon cancer cells [33], (ii) induction of apoptosis, as demonstrated in human breast cancer [31], choriocarcinoma [34] and prostate cancer cells [6] or (iii) the induction of re-differentiation, as observed in colon cancer cells as well [35]. In our present study, no signs of apoptosis after glitazone treatment could be observed by assessment of the nuclear morphology or by molecular analysis of DNA fragmentation. Therefore, G_1 arrest or induction of re-differentiation are more likely to be responsible for the observed anti-proliferative effect of glitazones in malignant melanoma. Thus, a combination of glitazones with an apoptosis-inducing agent, e.g. mistletoe lectin I, which has been proved to significantly induce apoptosis in malignant melanoma cells starting at very low concentrations [36], could have an additional anti-proliferative effect on melanoma cells. Ongoing studies will substantiate this promising outlook.

To investigate whether the anti-proliferative glitazone effect was indeed mediated by PPARγ activation, as determined by Brockman *et al.* using transfections and luciferase assays [33], PPARγ protein of all six cell lines was quantitatively measured on Western blots. Although PPARγ is known as a nuclear receptor, immunohistochemistry and Western blot analyses of cytoplasmic and nuclear proteins confirmed that PPARγ is predominantly located in the cytoplasm. Our findings corroborate the findings of Kim *et al.* who showed the PPARγ protein is predominantly located in the nucleus after ligand binding [37].

Quantitative analyses of PPARγ protein showed no correlation between the amount of the PPARγ protein and the respective susceptibility of the melanoma cell lines towards glitazones. Therefore, PPARγ agonist-mediated response may not depend only on the quantity of PPARγ protein present in a cell line, but on its stage of activation or on defects in factors necessary for PPARγ activation and binding to a PPARγ-responsive element. Furthermore, mutations of PPARγ in the melanoma cells could be a possible explanation. Palakurthi *et al.* postulated a PPARγ-independent anti-proliferative effect of glitazones [38]. It was demonstrated that glitazones showed an almost similar inhibition of cell proliferation of $PPAR\gamma^{-/-}$ and $PPAR\gamma^{+/+}$ mouse embryonic stem cells *in vitro*, independent of PPARγ. Taken together, it remains to be further analyzed whether activation of PPARγ or PPARγ-independent effects of PPARγ agonists contribute to the inhibition of cell growth.

In conclusion, this study demonstrates a significant inhibitory effect of glitazones on the proliferation of human melanoma cells *in vitro*. As glitazones are an already approved class of drugs for diabetes treatment, they might present a novel therapeutic approach in the treatment of human malignant melanoma.

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