Peroxisome proliferator-activated receptor-gamma ligands inhibit proliferation and induce apoptosis in mantle cell lymphoma

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Peroxisome proliferator-activated receptor-γ, a nuclear receptor and transcription factor, and its natural and synthetic ligands have become a focus of novel approaches to induction of apoptosis in solid tumors and hematologic malignancies, including malignant B-lineage cells. The effect on mantle cell lymphoma, a subtype with dismal prognosis, has not yet been analyzed. We investigated the effect of 15-deoxy-Δ-12,14-prostaglandin J_2 (15d-PGJ_2), pioglitazone (PGZ) or rosiglitazone (RGZ) on human mantle cell lymphoma cell lines (GRANTA-519, Hbl-2 and JeKo-1). Mantle cell lymphoma cell lines exhibited a high expression of Peroxisome proliferator-activated receptor-γ protein in Western blot analysis. MTT assays revealed anti-proliferative effects induced by both 15d-PGJ_2, the natural activator of Peroxisome proliferator-activated receptor-γ, and PGZ and RGZ, synthetic Peroxisome proliferator-activated receptor-γ ligands, in a dose-dependent manner. At a dose of 50 μmol/l, 15d-PGJ_2 induced growth inhibition in all cell lines. The anti-proliferative effect of PGZ and RGZ was slightly lower. Induction of apoptosis was indicated by annexin V staining. At a dose of 50 μmol/l, 15d-PGJ_2 led to apoptosis in all cell lines (87–99%) after 48 h of incubation. Again, the apoptotic effect with thiazolidinediones was slightly lower at the same dose level. This is the first study evaluating Peroxisome proliferator-activated receptor-γ expression and its therapeutic implications in human mantle cell lymphoma cells. Thiazolidinediones comprise anti-lymphoma activity *in vitro* and should be further explored for the treatment of mantle cell lymphoma. *Anti-Cancer Drugs* 17:763–769 © 2006 Lippincott Williams & Wilkins.

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Introduction

Mantle cell lymphoma is a subtype of non-Hodgkin's lymphoma that is characterized by an aggressive clinical course and a poor prognosis. Compared with other B cell lymphomas, patients with mantle cell lymphoma have a significantly shorter event-free and overall survival [1]. During the last decade, several attempts have been made to improve the outcome by novel treatment strategies [2–5]. Nevertheless, mantle cell lymphoma still remains an incurable disease and new treatment options are urgently needed.

15-Deoxy-Δ-12,14-prostaglandin J_2 (15d-PGJ_2), a terminal derivative of the PGJ_2 metabolism, is emerging as a potent anti-neoplastic agent. It is the biologically active metabolite of PGD_2, which is a major product of cyclooxygenase in bone marrow and macrophages, suggesting an additional role in immunological response [6,7]. Anti-cancer activity has been reported in a variety of tumor tissues *in vitro* and *in vivo*, including solid tumors such as colon, breast, lung and prostate cancer, as well as hematologic malignancies such as leukemia and multiple myeloma [8–14]. 15d-PGJ_2 is a natural activator of PPAR-γ and there is evidence that it exerts proliferation inhibitory effects on different tumor cells via PPAR-γ [9–11,13–15].

PPAR-γ is a transcription factor belonging to the nuclear receptor superfamily, which includes the retinoic acid receptors, the thyroid hormone receptors and steroid receptors. It binds to the promotor region of a target gene as heterodimer with the retinoid X receptor (RXR) and may stimulate transcription of target genes [16]. PPAR-γ or RXR ligands can activate this complex initiating transcription of target genes. Furthermore, PPAR-γ was shown to interact with other transcription factors such as Jun or nuclear factor-κB. In this manner, PPAR-γ may prevent them from binding to their response elements [8,17]. PPAR-γ is mainly expressed in normal adipocytes, adrenal gland, spleen, liver and activated macrophages,

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but recently it was discovered that PPAR-γ is expressed in tumor tissue as well [7,11]. Furthermore, the activation of PPAR-γ not only by the naturally occurring ligand, 15d-PGJ_2, but also by synthetic ligands, thiazolidinediones, has been shown to reduce tumor growth, interfere with tumor cell differentiation and induce apoptosis in a variety of human malignancies [9,11,13,15,18–21]. Thiazolidinediones, including troglitazone, rosiglitazone (RGZ) and pioglitazone (PGZ), comprise a group of synthetic PPAR-γ agonists that is currently in use for the treatment of type 2 diabetes mellitus and had revealed anti-tumor activity *in vitro* [11,22]. Troglitazone may cause idiosyncratic liver damage and its application has been discontinued, but pioglitazone and rosiglitazone are successfully applied in patients with diabetes mellitus.

Recently, it has been shown that human B lymphocytes, B lymphomas and multiple myeloma express PPAR-γ [9,23]. Influence of PPAR-γ agonists on cell growth of human mantle cell lymphoma cells, however, has not yet been analyzed. Our study addressed the proliferation inhibitory potential and the apoptosis induction of 15d-PGJ_2 and two thiazolidinediones, RGZ and PGZ, in human mantle cell lymphoma cell lines *in vitro*. First, expression of PPAR-γ in mantle cell lymphoma cells was analyzed by Western blot. Then, inhibition of proliferation and induction of apoptosis by the PPAR-γ ligands were investigated.

Material and methods

Cell culture

Two mantle cell lymphoma cell lines (GRANTA-519 and JeKo-1) were obtained from the DSMZ (Braunschweig, Germany). The mantle cell lymphoma cell line Hbl-2 was kindly provided by Dr Dreyling (Munich, Germany) [24,25]. All cell lines were maintained in continuous culture in RPMI 1640 (Biochrom, Berlin, Germany) supplemented by 100 units/ml penicillin, 100 μg/ml streptomycin and 10% fetal calf serum. Cell lines were cultured at 37°C in humidified 5% CO_2 atmosphere and were passaged three times weekly. The cell density was kept between 2×10^5 and 1×10^6/ml.

Chemicals

PGZ and RGZ were both dissolved at appropriate concentrations in dimethylsulfoxide and ethanol, and in cell culture medium at a final concentration of less than 10^{-3} mol/l. The synthetic ligands of PPAR-γ, PGZ and RGZ, were kindly provided by GlaxoSmithKline Pharmaceuticals (West Sussex, UK) and by Takeda Chemical Industries (Osaka, Japan), respectively. 15d-PGJ_2 was obtained from Calbiochem (San Diego, California, USA) and dissolved in ethanol. The solvents did not exceed 1% in the final solution. Aliquots of the stock solution were stored at −80°C.

Cell proliferation assay

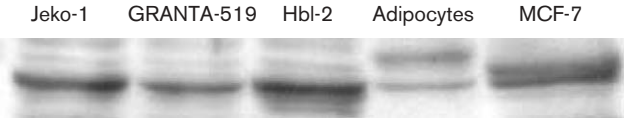
Mantle cell lymphoma cells were seeded in 96-well flat-bottom microtiter plates, at a cell density of 5×10^5 cells/ml. In dose–response studies, PPAR-γ ligands (PGZ, RGZ or 15d-PGJ_2) were added in different concentrations (10^{-4}, 5×10^{-5}, 10^{-5}, 5×10^{-6} and 10^{-6} mol/l). Each experimental condition was repeated at least in sextuplicate. The microtiter plate was incubated for 48 h, protected from light at 37°C, 5% CO_2 and 100% relative humidity. Proliferation was measured by cell proliferation kit I (MTT) (Roche Diagnostics, Mannheim, Germany). For the last 4 h of culture, cells were pulsed with 10 μl of the MTT labeling reagent at a final concentration of 0.5 mg/ml. This assay is based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by metabolic active cells [26]. To solubilize the crystals, a 100 μl of a solubilization solution containing 10% sodium dodecyl sulfate in 0.01 mol/l HCl was added into each well and the plate was allowed to stand overnight in the incubator in a humidified 37°C/5% CO_2 atmosphere. Finally, the absorbance was measured spectrophotometrically using a 550-nm wavelength ELISA reader and Anthos software (Anthos Labtec, Wals, Austria). Control experiments were performed with solvents of each PPAR-γ ligand alone, as well as in the absence of both the PPAR-γ ligands and their solvents. Solvents had no significant influence on cell proliferation.

Annexin V staining assay

In order to determine an apoptotic effect of PPAR-γ ligands on human mantle cell lymphoma cells, mantle cell lymphoma cell lines were exposed for 24, 48 and 72 h to 50 μmol/l PGZ, RGZ or 15d-PGJ_2. Apoptosis was measured by annexin V staining by using the annexin V–FITC kit (Bender MedSystems, Vienna, Austria). Cells (5×10^5/ml) were incubated in the presence or absence of PPAR-γ ligands in flat-bottom plates. They were centrifuged and washed twice with phosphate-buffered saline (PBS). The pellet was re-suspended in a 195 μl binding buffer contributed within the annexin V kit, spiked with 5 μl annexin V–FITC and incubated for 10 min at room temperature protected from light. After washing, the pellet of each sample was re-suspended once again in 190 ml binding buffer and 10 μl propidium iodide was added shortly before samples were analyzed by flow cytometry. All samples were analyzed on a FACS flow cytometer with an argon laser of 488 nm emission wavelength and the Cell Quest Pro software (Becton Dickinson, Heidelberg, Germany). As in the MTT assay, control experiments were performed with solvents, which had no significant influence on cell growth. Similar data were obtained in at least two independent experimental sets. The percentage of specific apoptosis was calculated as follows:

$$\frac{\text{experimental annexin V binding (\%)} - \text{control annexin V binding (\%)}}{100 - \text{control annexin V binding (\%)}} \times 100.$$

Fig. 1



Western blot analysis of the human mantle cell lymphoma cell lines (JeKo-1, GRANTA-519 and Hbl-2), all expressing peroxisome proliferator-activated receptor-γ (PPAR-γ) protein, a protein of 50 kDa. As positive control, a human adipocyte (AC) cell lysate as well as MCF-7, a breast cancer cell line with known expression of PPAR-γ, were used [21].

Analysis of the expression of cyclin D1

For the analysis of the expression of cyclin D1, cells were cultured in the presence or absence of 100 μmol/l RGZ, 100 μmol/l PGZ or 50 μmol/l 15d-PGJ_2 for 24 h. After that time, they were fixed and permeabilized using the IntraStain kit (Dako, Hamburg, Germany) according to the manufacturer's instructions. Cells were subsequently stained with a monoclonal mouse antibody against cyclin D1 (Santa Cruz Biotechnology, Heidelberg, Germany) at 1:100 dilution and a secondary phycoerythrin-conjugated F(ab′)2 fragment goat anti-mouse IgG antibody (Immunotech, Marseille, France). Cells were analyzed on a FACS flow cytometer.

Cell cycle analysis

Cell lines were exposed for 24, 48 and 72 h to 100 μmol/l RGZ, 100 μmol/l PGZ or 50 μmol/l 15d-PGJ_2, or solvent. Cells were collected, fixed with 70% ethanol, washed with PBS and treated for 15 min with solution B, containing 100 mg/l RNase, 3.4 mmol/l trisodium citrate, 0.1% Igepal, 1.5 mmol/l spermine tetrahydrochloride and 0.5 mmol/l tris(hydroxymethyl)aminomethane (dissolved in distilled water). Cells were then stained with solution C, containing 208 mg/l propidium iodide, 3.4 mmol/l trisodium citrate, 0.1% Igepal, 1.5 mmol/l spermine tetrahydrochloride and 0.5 mmol/l tris(hydroxymethyl) aminomethane (dissolved in distilled water), and incubated for 15 min in the dark. Finally, 20 000 cells were analyzed on a FACS flow cytometer (Becton Dickinson) using the Cell Quest Pro and ModFit software. All cell cycle experiments were reproduced once with similar results.

Western blot analysis

Expression of specific proteins was detected by Western blotting of centrifuged cells. First, protein concentrations of cell lysates were measured using the BCA protein assay kit (Perbio Science Deutschland, Bonn, Germany). Then, 50 μg of protein was separated by a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Munich, Germany), blocked in roti-block (Carl Roth, Karlsruhe, Germany) 10:1 dilution with aqua dest at room temperature for 90 min, and subsequently incubated with primary antibody for 2 h in roti-block 100:1 dilution with PBS/tween. As primary antibody, anti-PPAR-γ (sc-7196) was used (Santa Cruz, Heidelberg, Germany). After thoroughly washing the membrane, it was incubated with peroxidase-conjugated secondary antibody for 90 min. A signal was detected by chemoluminescence using the ECL detection system (Amersham Pharmacia Biotech, Freiburg, Germany). As an internal control, β-actin was detected with anti-β-actin antibodies (sc-1616) (Santa Cruz).

Statistical analysis

The data resulting from the MTT assay were presented as mean ± standard deviation. Each experimental condition was repeated at least in sextuplicate. Data from the annexin V staining assay were confirmed by at least two independent experiments.

Results

Expression of peroxisome proliferator-activated receptor-γ in mantle cell lymphoma cells

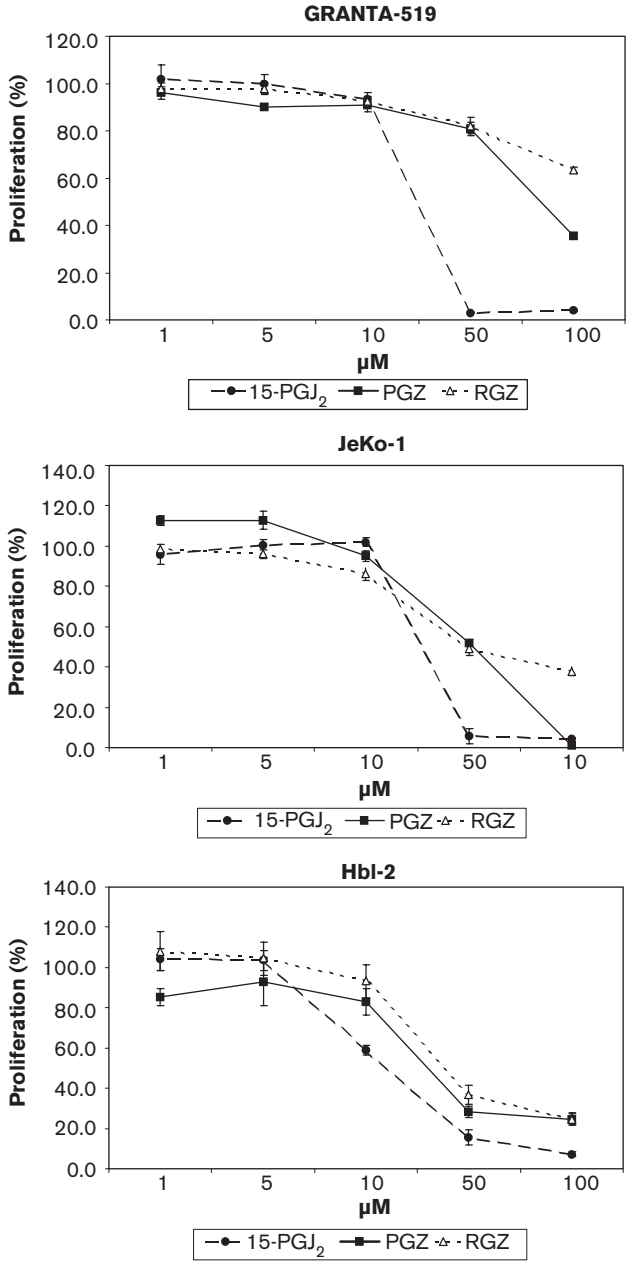
We tested the human mantle cell lymphoma cell lines GRANTA-519, JeKo-1 and Hbl-2. Expression of PPAR-γ protein was measured by Western blot analysis. Western blot analysis revealed a characteristic band with a molecular mass of 50 kDa representing PPAR-γ protein in all human mantle cell lymphoma cell lines tested as well as in MCF-7 cells, a breast cancer cell line, that had been proved to strongly express PPAR-γ protein [21]. As positive control, a human adipocyte cell lysate was used (Fig. 1).

Peroxisome proliferator-activated receptor-γ ligands induced proliferation inhibition

The MTT assay was used to determine the effect of two thiazolidinediones (PGZ and RGZ) and of a natural PPAR-γ agonist (15d-PGJ_2) on the proliferation of human mantle cell lymphoma cells. The MTT assay was carried out with the human mantle cell lymphoma cell lines GRANTA-519, JeKo-1 and Hbl-2. All three cell lines were sensitive to the PPAR-γ agonists. The growth inhibition was achieved in a dose-dependent manner.

PGZ induced significant inhibition of proliferation compared with controls in all cell lines tested. The growth inhibition was revealed as significant in doses

Fig. 2



Effect of the peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands PGZ, RGZ and 15d-PGJ_2 on the proliferation of mantle cell lymphoma cell lines. Cells were incubated for 48 h with either PGZ, RGZ or 15d-PGJ_2. Cell proliferation was measured by an MTT assay. Data were presented as a percentage of proliferation relative to untreated cells. PGZ, pioglitazone; RGZ, rosiglitazone; 15d-PGJ_2, 15-deoxy-Δ-12,14-prostaglandin J_2.

higher than 10 μmol/l. At a dose level of 50 μmol/l, cell proliferation was reduced in the MTT assay after 48 h of incubation to 52% in JeKo-1, 80% in GRANTA-519 and 28% in Hbl-2 cell lines (Fig. 2). RGZ significantly inhibited the proliferation of mantle cell lymphoma cells as well. The results were comparable to PGZ. Cell proliferation was reduced by 50 μmol/l RGZ after 48 h of incubation to 49% in JeKo-1, 82% in GRANTA-519 and 36% in Hbl-2 (Fig. 2).

The growth inhibition induced by 15d-PGJ_2 was much more pronounced than the inhibition induced by the synthetic PPAR-γ ligands, PGZ and RGZ. At a dose of 50 μmol/l cell proliferation was reduced to values between 5% in JeKo-1, 3% in GRANTA-519 and 16% in Hbl-2 cells (Fig. 2).

Peroxisome proliferator-activated receptor-γ agonists induced apoptosis in mantle cell lymphoma cell lines

To determine an apoptotic effect of 15d-PGJ_2 and the thiazolidinediones, RGZ and PGZ, on human mantle cell lymphoma cells, an annexin V staining assay was carried out. Phosphatidylserine externalization, a hallmark of early apoptosis, was quantified using annexin V bindings and FACS analysis. Cell lines were incubated with 50 μmol/l of PPAR-γ agonists, the lowest concentration that had been proven to be effective for growth inhibition in the MTT assay before. In the annexin V assay, 15-dPGJ_2 was more effective than PGZ and RGZ, corresponding to the data of the MTT assay. 15d-PGJ_2 strongly induced apoptosis in all cell lines tested. Specific apoptosis in mantle cell lymphoma cells ranged between 85 and 91% after 24 h of exposure to 15d-PGJ_2, and between 87 and 99% after 48 h. Apoptosis induced by PGZ and RGZ was less pronounced. In JeKo-1 and Hbl-2 cells, apoptosis rose with time. After 24 h of exposure to RGZ or PGZ, specific apoptosis was low in both cell lines. After 48 h, however, specific apoptosis ranged between 48 and 55% for cells exposed to PGZ, and between 42 and 64% for cells exposed to RGZ. As already detected in the MTT assay, GRANTA-519 were less vulnerable to exposure to PGZ or RGZ. After 24 h of exposure to PGZ or RGZ, specific apoptosis was 29 and 34%, respectively, and after 48 h, specific apoptosis was determined to be 18 and 8%, respectively (Table 1).

Peroxisome proliferator-activated receptor-γ agonists induced a decline in cyclin D1 expression in mantle cell lymphoma cell lines

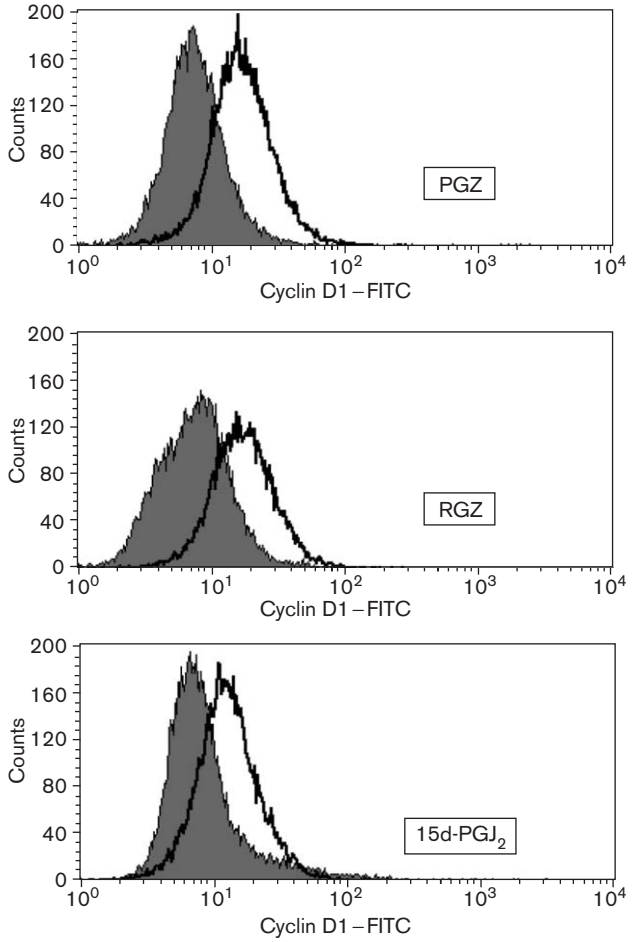
The ability of PPAR-γ agonists to influence cyclin D1 expression in JeKo-1 cells was analyzed on a FACS flow

Table 1 Apoptosis induction by the peroxisome proliferator-activated receptor-γ ligands PGZ, RGZ or 15d-PGJ_2 in mantle cell lymphoma cells

Cell line	Specific apoptosis after treatment (%)					
	PGZ		RGZ		15d-PGJ_2	
Incubation time (h)	24	48	24	48	24	48
JeKo-1	9	55	10	64	84	99
GRANTA-519	29	18	34	8	91	93
Hbl-2	1	42	1	42	65	87

Cells were incubated for 24 and 48 h with either PGZ, RGZ or 15d-PGJ2. Rate of specific apoptosis in different cell lines is presented. PGZ, pioglitazone; RGZ, rosiglitazone; 15d-PGJ_2, 15-deoxy-Δ-12,14-prostaglandin J_2.

Fig. 3



Expression of cyclin D1 after treatment with peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands detected by flow cytometry. JeKo-1 cells were incubated for 24 h in the presence of PGZ, RGZ or 15d-PGJ_2 (gray area under the curve). As controls, JeKo-1 cells were incubated with solvent only (uncolored area under the curve). Incubation with PPAR-γ ligands revealed a reduction of cyclin D1 expression. PGZ, pioglitazone; RGZ, rosiglitazone; 15d-PGJ_2, 15-deoxy-Δ-12,14-prostaglandin J_2.

cytometer. We analyzed the effect at a concentration of PPAR-γ agonists that had been proven to induce apoptosis after 48 h, but the incubation time was shortened avoiding complete apoptosis. For 24 h, JeKo-1 cells were exposed to PGZ, RGZ or 15d-PGJ_2. The inhibition of proliferation was accompanied by markedly decreased cyclin D1 expression in this mantle cell lymphoma cell line (Fig. 3).

Peroxisome proliferator-activated receptor-γ agonists did not inhibit cell cycle progression

The ability of PPAR-γ agonists to inhibit cell cycle progression was analyzed by FACS analysis with two of the three mantle cell lymphoma cell lines (JeKo-1 cells and Hbl-2 cells). One potential target of cellular growth inhibitory activity of PPAR-γ agonists involves the cell cycle. In hematological malignancies, cell cycle analysis had revealed cell cycle arrest in some of the cell lines tested with PPAR-γ agonists [10,20]. We performed cell cycle analysis after 24, 48 and 72 h. We did not find any reproducible increase in the G_0/G_1 phase or a corresponding arrest in G_2/M after exposition to RGZ, PGZ or 15D-PGJ_2, neither in JeKo-1 cells nor in Hbl-2 cells. All experiments were performed at least twice.

Discussion

The anti-cancer activity of PPAR-γ ligands has been demonstrated in a number of investigations *in vitro* and in animal models of cancer [9,11,13,14,18–21,27]. Both the natural activator 15d-PGJ_2 and the thiazolidinediones exhibited anti-cancer activity to a different extent. For a variety of tumor tissue, the expression of PPAR-γ has been shown [9,14,19,28–30]. For B cells, PPAR-γ protein was expressed in different cell lines that reflected stages of B cell ontogeny [28]. Furthermore, PPAR-γ protein expression was detected in CLL cell lineages [29]. None of the cell lines tested, however, were mantle cell lymphoma cell lines. In the present study, we found that PPAR-γ protein is strongly expressed in three B cell lineages from human mantle cell lymphoma. These data are in line with those obtained in B cell lines using reverse transcriptase polymerase chain reaction, Western blot analysis and immunohistochemistry. In our study, we could not detect any correlation of PPAR-γ protein expression of mantle cell lymphoma cells and the proliferation inhibition in these three cell lines. Theoretically, the response to PPAR-γ ligands might be influenced by the quantitative expression of PPAR-γ in different tissues. In B cell lines of various differentiation statuses, however, the relative amount of PPAR-γ protein did not vary significantly [28,29].

We found a strong proliferation inhibition induced by 15d-PGJ_2 beginning at concentrations of 10 μmol/l and leading to nearly complete inhibition at 50 μmol/l. For thiazolidinediones, this effect was slightly lower. These data are in line with the ones published for multiple myeloma cells or human B lymphocytic leukemia cells [9,20]. To confirm that proliferation inhibition of human mantle cell lymphoma cells observed in the MTT assay was due to apoptosis, we carried out an annexin V staining assay. Both 15d-PGJ_2 and thiazolidinediones induced apoptosis in mantle cell lymphoma cells. 15d-PGJ_2 was revealed to be more effective than PGZ and RGZ at similar doses (5×10^{-5} mol/l). In all test systems, we found that the response of mantle cell lymphoma cells to the treatment with the natural PPAR-γ ligand, 15d-PGJ_2, was stronger than that to treatment with thiazolidinediones. In B cells, the same phenomenon was observed in all studies comparing the natural and the synthetic ligands [9,23,31].

In several studies, it was postulated that tumor cells treated with PPAR-γ agonists were killed in an apoptotic fashion even though the precise pathway by which PPAR-γ agonists lead to apoptosis remains unknown [28,31]. At least some explanations exist, however. PPAR-γ ligands appear to induce apoptosis by inducing a G_1 to S cell cycle arrest, by a loss of mitochondrial membrane, and by caspase 2 and 3 activation [10,31]. In macrophages, the treatment with PPAR-γ agonists inhibited their activation and differentiation. Furthermore, PPAR-γ agonists inhibited STAT and nuclear factor-κB activities, both members of transcription factor families, which are also involved in cell proliferation and cell survival of tumor cells [7]. In mantle cell lymphoma, cyclin D1 is a constitutionally up-regulated protein that is involved in the cell proliferation. We found that in mantle cell lymphoma cells (JeKo-1) all PPAR-γ agonists tested repress cyclin D1 expression. On the other hand, a cell cycle arrest in the G_1/G_0 phase, as indicated in the literature for some cell lines, could not be demonstrated in JeKo-1 and Hbl-2 cells. The increase in the G_0/G_1 phase in lymphatic cells or leukemia cells, however, was only inconstantly shown in the literature [10,20]. Only a part of the cell lines tested revealed a cell cycle arrest even though induction of apoptosis and proliferation inhibition by PPAR-γ agonists was evident in these cells. Furthermore, it should be critically stated that the observation of a repression of cyclin D1 is not proved to be the major mechanism responsible for apoptosis in these cells [32]. In some other investigations, the correlation of growth inhibition potential of thiazolidinediones and PPAR-γ expression is illuminated in a critical way as well. In mouse embryonal stem cells, the growth-inhibiting effect of troglitazone was not correlated with PPAR-γ expression and anti-tumor activity was not attributed to PPAR-γ activation, but to translation initiation and G_1 arrest. Even the repression of cyclin D1, which was observed after treatment with troglitazone, was not attributed to PPAR-γ in that mouse model [33]. The repression of cyclin D1 might be mediated at a posttranscriptional level as the mRNA level remained unaltered after treatment with troglitazone [32,33].

Nevertheless, especially in B cells, all investigations suggest that anti-cancer effect is mediated by PPAR-γ. The use of PPAR-γ antagonists was able to inhibit significantly all effects induced by PPAR-γ agonists in different B cells. In myeloma cells, transfection of myeloma cells with a PPAR-γ mutant inhibited the transcriptional inactivation of STAT3 by PPAR-γ ligands compared with PPAR-γ wild-type [29,34]. Furthermore, the RXR ligand, 9-*cis*-retinoic acid, in combination with PPAR-γ ligands significantly enhanced the apoptosis in B cells, suggesting that the downstream pathway of the RXR/PPAR-γ transcription factor is responsible for the effect [31]. Preliminarily, one can conclude that in some tissue anti-proliferative activity of PPAR-γ ligands might not be exclusively mediated via PPAR-γ, but that there might be some other mechanisms involved that are independent of PPAR-γ. On the other hand, however, there is strong evidence that the natural and synthetic PPAR-γ ligands, 15d-PGJ_2 and thiazolidinediones, exert proliferation inhibitory effects on different tumor cells via PPAR-γ, and these data are underlined by the present study [9,11,13–15].

Conclusion

The present study shows that mantle cell lymphoma cells express PPAR-γ protein, representing a potential target for anti-cancer treatment. The natural PPAR-γ ligand, 15d-PGJ_2, as well as the thiazolidinediones, RGZ and PGZ, exhibit a high anti-lymphoma activity *in vitro* in mantle cell lymphoma cells. Furthermore, it revealed that PPAR-γ ligands induce apoptosis in mantle cell lymphoma cells, even though the exact pathway still remains unknown. These ligands may have a synergistic effect in combination with other agents with anti-lymphoma activity. Therefore, PPAR-γ ligands might contribute to the treatment of mantle cell lymphoma. This should be investigated in further studies.

References

1 Hiddemann W, Unterhalt M, Herrmann R, Woltjen HH, Kreuser ED, Trumper L, *et al.* Mantle-cell lymphomas have more widespread disease and a slower response to chemotherapy compared with follicle-center lymphomas: results of a prospective comparative analysis of the German Low-Grade Lymphoma Study Group. *J Clin Oncol* 1998; **16**:1922–1930.

2 Goy A, Younes A, McLaughlin P, Pro B, Romaguera JE, Hagemeister F, *et al.* Phase II study of proteasome inhibitor bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. *J Clin Oncol* 2005; **23**:667–675.

3 Lenz G, Dreyling M, Hoster E, Wormann B, Duhrsen U, Metzner B, *et al.* Immunochemotherapy with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone significantly improves response and time to treatment failure, but not long-term outcome in patients with previously untreated mantle cell lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). *J Clin Oncol* 2005; **23**:1984–1992.

4 Zavrski I, Jakob C, Schmid P, Krebbel H, Kaiser M, Fleissner C, *et al.* Proteasome: an emerging target for cancer therapy. *Anticancer Drugs* 2005; **23**:475–481.

5 Heider U, Kaiser M, Sterz J, Zavrski I, Jakob C, Fleissner C, *et al.* Histone deacetylase inhibitors reduce VEGF production and induce growth suppression and apoptosis in human mantle cell lymphoma. *Eur J Haematol* 2006; **76**:42–50.

6 Padilla J, Kaur K, Harris SG, Phipps RP. PPAR-γ-mediated regulation of normal and malignant B lineage cells. *Ann N Y Acad Sci* 2000; **905**:97–109.

7 Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature* 1998; **391**:79–82.

8 Piva R, Gianferretti P, Ciucci A, Taulli R, Belardo G, Santoro MG. 15-Deoxy-delta 12,14-prostaglandin J_2 induces apoptosis in human malignant B cells: an effect associated with inhibition of NF-kappa B activity and down-regulation of antiapoptotic proteins. *Blood* 2005; **105**:1750–1758.

9 Eucker J, Bangeroth K, Zavrski I, Krebbel H, Zang C, Heider U, *et al.* Ligands of peroxisome proliferator-activated receptor gamma induce apoptosis in multiple myeloma. *Anticancer Drugs* 2004; **15**:955–960.

10 Zang C, Liu H, Posch MG, Waechter M, Facklam M, Fenner MH, *et al.* Peroxisome proliferator-activated receptor gamma ligands induce growth inhibition and apoptosis of human B lymphocytic leukemia. *Leuk Res* 2004; **28**:387–397.

11 Koeffler HP. Peroxisome proliferator-activated receptor γ and cancers. *Clin Cancer Res* 2003; **9**:1–9.

12 Liu H, Zang C, Fenner MH, Possinger K, Elstner E. PPAR gamma ligands and ATRA inhibit the invasion of human breast cancer cells *in vitro*. *Breast Cancer Res Treat* 2003; **79**:63–74.

13 Smith MR, Kantoff PW. Peroxisome proliferator activated receptor gamma (PPARγ) as a novel target for prostate cancer. *Invest New Drugs* 2002; **20**:195–200.

14 Sarraf P, Mueller E, Jones D, King FJ, DeAngelo DJ, Partridge JB, *et al.* Differentiation and reversal of malignant changes in colon cancer through PPARγ. *Nat Med* 1998; **4**:1046–1052.

15 Zander T, Kraus JA, Grommes C, Schlegel U, Feinstein D, Klockgether T, *et al.* Induction of apoptosis in human and rat glioma by agonists of the nuclear receptor PPARγ. *J Neurochem* 2002; **81**:1052–1060.

16 Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 1999; **20**:649–688.

17 Konopleva M, Andreeff M. Role of peroxisome proliferator-activated receptor-gamma in hematologic malignancies. *Curr Opin Hematol* 2002; **9**:294–302.

18 Elstner E, Williamson EA, Zang C, Fritz J, Heber D, Fenner M, *et al.* Novel therapeutic approach: ligands for PPARγ and retinoid receptors induce apoptosis in *bcl-2*-positive human breast cancer cells. *Breast Cancer Res Treat* 2002; **74**:155–165.

19 Toyoda M, Takagi H, Horiguchi N, Kakizaki S, Sato K, Takayama H, *et al.* A ligand for peroxisome proliferator activated receptor gamma inhibits cell growth and induces apoptosis in human liver cancer cells. *Gut* 2002; **50**:563–567.

20 Asou H, Verbeek W, Williamson E, Elstner E, Kubota T, Kamada N, *et al.* Growth inhibition of myeloid leukemia cells by troglitazone, a ligand for peroxisome proliferator activated receptor gamma, and retinoids. *Int J Oncol* 1999; **15**:1027–1031.

21 Elstner E, Muller C, Koshizuka K, Williamson EA, Park D, Asou H, *et al.* Ligands for peroxisome proliferator-activated receptor gamma and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells *in vitro* and in BNX mice. *Proc Natl Acad Sci U S A* 1998; **95**: 8806–8888.

22 Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM. A prostaglandin J2 metabolite binds peroxisome proliferator activated receptor γ and promotes adipocyte differentiation. *Cell* 1995; **83**:813–819.

23 Padilla J, Leung E, Phipps RP. Human B lymphocytes and B lymphomas express PPAR-gamma and are killed by PPAR-γ agonists. *Clin Immunol* 2002; **103**:22–33.

24 Abe M, Nozawa Y, Wakasa H, Ohno H, Fukuhara S. Characterization and comparison of two newly established Epstein–Barr virus-negative lymphoma B-cell lines. Surface markers, growth characteristics, cytogenetics, and transplantability. *Cancer* 1988; **61**:483–490.

25 de Leeuw RJ, Davies JJ, Rosenwald A, Bebb G, Gascoyne RD, Dyer MJ, *et al.* Comprehensive whole genome array CGH profiling of mantle cell lymphoma model genomes. *Hum Mol Genet* 2004; **13**:1827–1837.

26 Maehara Y, Anai H, Tamada R, Sugimachi K. The ATP assay is more sensitive than the succinate dehydrogenase inhibition test for predicting cell viability. *Eur J Cancer Clin Oncol* 1987; **23**:273–276.

27 Kubota T, Koshizuka K, Williamson EA, Asou H, Said JW, Holden S, *et al.* Ligand for peroxisome proliferator-activated receptor gamma (troglitazone) has potent antitumor effect against human prostate cancer both *in vitro* and *in vivo*. *Cancer Res* 1998; **58**:3344–3352.

28 Padilla J, Kaur K, Cao HJ, Smith TJ, Phipps RP. Peroxisome proliferator activator receptor-γ agonists and 15-deoxy-Δ12,14-PGJ_2 induce apoptosis in normal and malignant B-lineage cells. *J Immunol* 2000; **165**:6941–6948.

29 Konopleva M, Elstner E, McQueen TJ, Tsao T, Sudarikov A, Hu W, *et al.* Peroxisome proliferator-activated receptor gamma and retinoid X receptor ligands are potent inducers of differentiation and apoptosis in leukemias. *Mol Cancer Ther* 2004; **3**:1249–1262.

30 Zang C, Wachter M, Liu H, Posch MG, Fenner MH, Stadelmann C, *et al.* Ligands for PPARγ and RAR cause induction of growth inhibition and apoptosis in human glioblastomas. *J Neurooncol* 2003; **65**:107–118.

31 Ray DM, Bernstein SH, Phipps RP. Human multiple myeloma cells express peroxisome proliferator-activated receptor gamma and undergo apoptosis upon exposure to PPAR gamma ligands. *Clin Immunol* 2004; **113**:203–213.

32 Huang JW, Shiau CW, Yang YT, Kulp SK, Chen KF, Brueggmeier RW, *et al.* Peroxisome proliferator-activated receptor gamma-independent ablation of cyclin D1 by thiazolidinediones and their derivatives in breast cancer cells. *Mol Pharmacol* 2005; **67**:1342–1348.

33 Palakurthi SS, Aktas H, Grubissich LM, Mortensen RM, Halperin JA. Anticancer effects of thiazolidinediones are independent of peroxisome proliferator-activated receptor gamma and mediated by inhibition of translation initiation. *Cancer Res* 2001; **61**:6213–6218.

34 Wang LH, Yang XY, Zhang X, Huang J, Hou J, Li J, *et al.* Transcriptional inactivation of STAT3 by PPAR gamma suppresses IL-6-responsive multiple myeloma cells. *Immunity* 2004; **20**:205–218.