TREATMENT OF LIPOSARCOMAS USING A COMBINATION OF THIAZOLIDINEDIONES AND RETINOID X RECEPTOR SELECTIVE AGONISTS

Inventors: Ronald M. Evans, La Jolla; Peter Tontonoz, Los Angeles, both of CA (US); Bruce M. Spiegelman, Waban, MA (US); Barry M. Forman, Newport Beach, CA (US)

Assignee: The Salk Institute for Biological Studies, La Jolla, CA (US)

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Primary Examiner—Edward J. Webman
Assistant Examiner—Helen Nguyen
Attorney, Agent or Firm—Stephen E. Reiter; Foley & Lardner

ABSTRACT

In accordance with the present invention, it has been discovered that PPARγ is expressed consistently in each of the major histologic types of human liposarcoma. It has further been discovered that maximal activation of PPARγ with exogenous ligand (a thiazolidinedione or derivative thereof) promotes terminal differentiation of primary human liposarcoma cells. It has still further been discovered that RXR-specific ligands are also potent adipogenic agents in cells expressing the PPARγ/RXRs heterodimer, and that simultaneous treatment of liposarcoma cells with a thiazolidinedione moiety (a PPARγ-selective class of compounds) and an RXR-specific ligand results in an additive stimulation of differentiation. Accordingly, according to the invention, there have been identified compositions which are useful for the treatment of liposarcomas.

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This application is 371 of PCT/US97/24187 filed Dec. 29, 1997, which claims benefit of Ser. No. 60/034,133 filed Dec. 31, 1996.

FIELD OF THE INVENTION

The present invention relates to methods for the treatment of liposarcomas. In another aspect, the present invention relates to compounds and compositions which are useful for carrying out the above-referenced methods.

BACKGROUND OF THE INVENTION

Liposarcoma is the most common soft tissue malignancy in adults, accounting for at least 20% of all sarcomas in this age group. Multiple histologic subtypes of liposarcoma are recognized, including well differentiated, de-differentiated, myxoid, round cell and pleomorphic. The histologic subtype is predictive of both the clinical course of the disease and the ultimate prognosis.

Localized disease is treated primarily with surgery, often in combination with radiotherapy. Metastatic liposarcoma is associated with an extremely poor prognosis, with average five year survival ranging from 70% to 25%, depending on the type of tumor. Unfortunately, conventional chemotherapy for metastatic liposarcoma leads to complete response in only about 10% of cases. Thus, for most patients, conventional chemotherapy is largely palliative.

Induction of terminal differentiation represents a promising alternative to conventional chemotherapy for certain malignancies. For example, the retinoic acid receptor alpha (RARα), which plays an important role in the differentiation and malignant transformation of cells of myelocytic lineage, has been used as a target for intervention in acute promyelocytic leukemia. Indeed, differentiation therapy with all-trans retinoic acid has become the standard of care for this disease. In view of this success, it has been speculated that nuclear receptors that regulate growth and differentiation of other cell types may also represent potential targets for differentiation therapy.

Recent years have seen important advances in the understanding of the molecular basis of adipocyte differentiation. Central to this process is the induction of the adipocyte-selective nuclear receptor, peroxisome proliferator-activated receptor gamma (PPARγ). This receptor and its heterodimeric partner, the retinoid X receptor alpha (RXRα), form a DNA binding complex that regulates transcription of adipocyte-specific genes. Expression and activation of PPARγ in fibroblastic cell lines triggers the adipocyte gene expression cascade and leads to development of the adipocyte phenotype.

Accordingly, the development of effective, non-invasive methods for treating liposarcomas would represent a significant advancement in the therapeutic arts.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have discovered that PPARγ is expressed consistently in each of the major histologic types of human liposarcoma. It has further been discovered that maximal activation of PPARγ with exogenous ligand (a thiazolidinedione or derivative thereof) promotes terminal differentiation of primary human liposarcoma cells. It has still further been discovered that RXR-specific ligands are also potent adipogenic agents in cells expressing the PPARγ/RXRα heterodimer, and that simultaneous treatment of liposarcoma cells with a thiazolidinedione moiety (a PPARγ-selective class of compounds) and an RXR-specific ligand results in an additive stimulation of differentiation. Accordingly, according to the invention, there have been identified compositions which are useful for the treatment of liposarcomas.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for the treatment of liposarcomas, said method comprising administering to a subject in need of such treatment an amount of a therapeutic composition effective to ameliorate the effects of neoplastic cell proliferation of liposarcoma cells, wherein said therapeutic composition comprises at least one thiazolidinedione and at least one retinoid X receptor (RXR) selective agonist in a pharmaceutically acceptable carrier therefor. Invention methods can also be used in a prophylactic manner, i.e., to prevent the onset of liposarcoma.

Thiazolidinediones contemplated for use in the practice of the present invention can be described broadly with reference to the general structure I:
(alkylene oxide), substituted poly(alkylene oxide); poly(alkylene sulfide), substituted poly(alkylene sulfide), poly(alkylene amine) or substituted poly(alkylene amine); with R₃ having in the range of 1 up to about 15 carbon atoms being preferred;

R₄ is hydrogen, hydroxy, halogen, alkoyl, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl; with R₄ having in the range of 0 up to about 6 carbon atoms being preferred;

R₅ is hydrogen, formyl, acyl, lower alkyl, substituted lower alkyl or a thiazolidinedionyl moiety; with R₅ having in the range of 0 up to about 4 carbon atoms being preferred;

R₆ is hydrogen, hydroxy, lower alkoyl, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen; with R₆ having in the range of 0 up to about 6 carbon atoms being preferred and

R₇ is hydrogen, hydroxy, lower alkoyl, lower alkyl, substituted lower alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen; with R₇ having in the range of 0 up to about 6 carbon atoms being preferred.

Those of skill in the art recognize that the core ring of structure I can be any one of a number of different aromatic or pseudo-aromatic structures, e.g., a benzene ring, a pyridine ring, a pyrazine, an oxazine, and the like.

As employed herein, “lower alkyl” refers to straight or branched chain alkyl groups having in the range of about 1 up to 4 carbon atoms; “alkyl” refers to straight or branched chain alkyl groups having in the range of about 1 up to 12 carbon atoms; “substituted alkyl” refers to alkyl groups further bearing one or more substituents such as hydroxy, alkoxyl (of a lower alkyl group), mercapto (of a lower alkyl group), halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide, heteroatom-containing cyclic moieties, substituted heteroatom-containing cyclic moieties, and the like.

As employed herein, “alkenyl” refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and having in the range of about 2 up to 12 carbon atoms and “substituted alkyl” refers to alkyl groups further bearing one or more substituents as set forth above.

As employed herein, “alkynyl” refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon triple bond, and having in the range of about 2 up to 12 carbon atoms, and “substituted alkynyl” refers to alkynyl groups further bearing one or more substituents as set forth above.

As employed herein, “aryl” refers to aromatic groups having in the range of 6 up to 14 carbon atoms and “substituted aryl” refers to aryl groups further bearing one or more substituents as set forth above.

As employed herein, “alkylaryl” refers to alkyl-substituted aryl groups and “substituted alkylaryl” refers to alkylaryl groups further bearing one or more substituents as set forth above.

As employed herein, “alkenylaryl” refers to alkenyl-substituted aryl groups and “substituted alkenylaryl” refers to alkenylaryl groups further bearing one or more substituents as set forth above.

As employed herein, “alkynylaryl” refers to alkynyl-substituted aryl groups and “substituted alkynylaryl” refers to alkynylaryl groups further bearing one or more substituents as set forth above.

As employed herein, “aryalkyl” refers to aryl-substituted alkyl groups and “substituted arylalkyl” refers to arylalkyl groups further bearing one or more substituents as set forth above.

As employed herein, “aryalkenyl” refers to aryl-substituted alkenyl groups and “substituted arylalkenyl” refers to arylalkenyl groups further bearing one or more substituents as set forth above.

As employed herein, “aryalkynyl” refers to aryl-substituted alkynyl groups and “substituted arylalkynyl” refers to arylalkynyl groups further bearing one or more substituents as set forth above.

As employed herein, “poly(alkylene oxide)” refers to compounds having the general structure:

\[-(CR₃)ₙ-OH,\]

wherein each R₃ is independently selected from hydrogen or lower alkyl, x falls in the range of 1 up to about 4 and y falls in the range of 2 up to about 8; “substituted poly(alkylene oxide)” refers to poly(alkylene oxide) groups further bearing one or more substituents as set forth above.

As employed herein, “poly(alkylene sulfide)” refers to compounds having the general structure:

\[-(CR₄)ₙ-S-Sₙ-\]

wherein R₄, X and Y are as defined above; “substituted poly(alkylene sulfide)” refers to poly(alkylene sulfide) groups further bearing one or more substituents as set forth above.

As employed herein, “poly(alkylene amine)” refers to compounds having the general structure:

\[-(CR₅)ₙ-N(R₆)ₙ-\]

wherein R₅, X and Y are as defined above; “substituted poly(alkylene amine)” refers to poly(alkylene amine) groups further bearing one or more substituents as set forth above.

As employed herein, “acyl” refers to alkyl-carbonyl species.

As employed herein, “halogen” or “halo” refers to fluoro substituents, chloro substituents, and bromo substituents or iodo substituents.

In a presently preferred aspect of the present invention, “A” of Formula I has the structure:

\[-Y₋(CHₓ₋₂₋)₋Z,\]

wherein:

Y is —O— or —S—,

n is 0 or 1,

x falls in the range of 2 up to 12; and

Z is a thiazolidinedionyl moiety.

In another preferred aspect of the present invention, “Rₓ” of Formula I is methyl, ethyl, propyl, butyl, n-propyl, n-butyI, ethoxy, propoxy, butoxy, and the like.

In yet another preferred aspect of the present invention, “Rₓ” of Formula I is hydrogen, hydroxy, alkoxyl, and the like.

In still another preferred aspect of the present invention, “Rₓ” of Formula I is selected from formyl, acyl, a thiazolidinedionyl moiety, and the like.

In a still further preferred aspect of the present invention, “Rₓ” of Formula I is hydrogen.

In yet another preferred aspect of the present invention, at least one of R₂, R₃, R₄, R₅ and R₆ (in addition to A) is not
hydrogen. It is especially preferred that at least two of R₂, R₃, R₅, R₆, and R₇ (in addition to A) are not hydrogen. A plurality of substituents on the ring of structure I is especially preferred when m or the sum of (m+n), with reference to the backbone of A, is less than or equal to 6.

Presently preferred species contemplated for use in the practice of the present invention include compounds wherein:

A is a thiazolidinedione moiety (e.g., —O—(CH₂)₇—thiazolidenedione, wherein y falls in the range of about 2 to 8),

R₂ is hydrogen or lower alkyl,

R₃ is hydroxy or alkoxy,

R₅ is acyl or a thiazolidinedione moiety; and

R₆ and R₇ are each hydrogen.

The above-described compounds can be readily prepared using a variety of synthetic methods, as are well known by those of skill in the art. For example, many of the above-described compounds can be prepared chemically or enzymatically.

RXR selective ligands contemplated for use in the practice of the present invention include substituted benzoxa derivatives thereof (e.g., substituted benzoates), substituted nitocic acids or derivatives thereof (e.g., substituted nitocinates), substituted carbonylated furans, and the like. Exemplary agonists contemplated for use herein include 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, 1,3-propylene glycol ketal of 4-[1-(5,5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, methyl 4-[1-(3,8,8-trimethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)carbonyl]benzoate, methyl 4-[1-(3,5,5-trimethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)carbonyl]benzoate, methyl 4-[1-(1,1,2,3,3,6-hexamethyldi-n-yl)carbonyl]benzoate, methyl 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)carbonyl]nicotinate, methyl 4-[1-(3,8,8-trimethyl-5,6,7,8-tetrahydro-2-naphthyl-2-yl)ethenyl]benzoate, methyl 4-[1-(1,1,2,3,3,6-hexamethyldi-n-yl)ethenyl]benzoic acid, methyl 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethenyl]nicotinate, 4-[1-(3,8,8-trimethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethenyl]benzoic acid, 4-[1-(3,5,5-trimethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethenyl]nicotinic acid, methyl 4-[1-methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethyl]benzoate, 4-[2-methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethyl]benzoic acid, 4-[1-methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethyl]benzoic acid, 4-[1-methyl-1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethyl]nicotinamide, methyl 4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)cyclopropyl]benzoate, methyl 4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)cyclopropyl]nicotinate, methyl 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)cyclopropyl]benzoate, methyl 4-[2-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)cyclopropyl]nicotinic acid, 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)cyclopropyl]nicotinic acid (also referred to in the art as “LIG268”), 3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-ol, methyl 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethyl]benzoate, methyl 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)oxy]benzoate, 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)oxy]benzoic acid, methyl 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)carbonyl]benzoate, methyl 3-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)carbonyl]benzoic acid, 3-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)carbonyl]benzoic acid, the carboxylated furan derivative referred to as AGN191701 (see Mol. and Cell. Biol. 15:3540-3551 (1995)), and the like.

Presently preferred RXR selective agonists contemplated for use in vivo include 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)cyclopropyl]nicotinic acid and 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethenyl]benzoic acid.

Combinations of thiazolidinediones with RXR selective agonists contemplated for use in the practice of the present invention can be employed for both in vitro and in vivo applications. For in vivo applications, the above-described compositions can be incorporated into a pharmaceutically acceptable formulation for administration. Those of skill in the art can readily determine suitable dosage levels when compositions contemplated for use in the practice of the present invention are so used.

In accordance with another embodiment of the present invention, there are provided compositions comprising at least one thiazolidenedione and at least one retinoic acid receptor (RXR) selective agonist, wherein said thiazolidenedione has the structure I, as described hereinabove.

In accordance with a particular embodiment of the present invention, compositions comprising at least one thiazolidenedione, an agonist of RXR, and a pharmaceutically acceptable carrier are also contemplated. Exemplary pharmaceutically acceptable carriers include carriers suitable for oral, intravenous, subcutaneous, intramuscular, intracutaneous, and the like administration. Administration in the form of creams, lotions, tablets, dispersible powders, granules, syrups, elixirs, sterile aqueous or non-aqueous solutions, suspensions or emulsions, and the like, is contemplated.

For the preparation of oral liquids, suitable carriers include emulsions, solutions, suspensions, syrups, and the like, optionally containing additives such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents, and the like.

For the preparation of fluids for parenteral administration, suitable carriers include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized, for example, by filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, or by heating the compositions. They can also be manufactured in the form of sterile water, or some other sterile injectable medium immediately before use.

PPARγ is expressed at high levels in the adipose tissues of mouse and rat (see, for example, Tontonoz et al., in Genes Dev. 8:1224-1234 (1994) and Braissant et al., in Endocrinology 137:354-366(1996)). To determine the tissue distri-
bution of this receptor in humans. Northern analysis of RNA prepared from a variety of human tissues was performed. Human PPARγ is seen to be expressed at highest levels in adipose tissue and at lower levels in several other tissues including lung and kidney. As a control, the blot was also hybridized with cDNA for the adipocyte-specific binding protein αP2. The heart and muscle samples can be seen to contain small amounts of αP2 mRNA, suggesting that these tissues also contain some adipose cells.

Tumorigenesis frequently involves the inactivation or downregulation of genes responsible for initiating and maintaining a differentiated phenotype. As PPARγ appears to play a central role in the adipocytic differentiation process, the expression of this receptor was examined in a series of human liposarcomas. This series included RNA prepared from each of the three major histologic subtypes of liposarcoma; well differentiated/dedifferentiated, myxoid/round cell and pleomorphic. The histologic and cytogenetic characteristics of each tumor is given in Table 3 (see Example 2). For the most part, the well differentiated/dedifferentiated tumors exhibited ring chromosomes and giant marker chromosomes, the myxoid/round cell liposarcomas exhibited the characteristic (13;14) (13q14;14q32) translocation, and the pleomorphic forms exhibited complex rearrangements. Surprisingly, despite their block in differentiation, each liposarcoma examined was found to express high levels of PPARγ RNA, comparable to that of normal fat. These results suggest that most if not all liposarcomas have been transformed at a point in the differentiation process after induction of PPARγ expression. In contrast, PPARγ RNA was not expressed at significant levels in any other type of soft tissue sarcoma examined, including leiomyosarcoma (n=4), fibrosarcoma (n=3), angiosarcoma (n=2), malignant peripheral nerve sheath tumor (MPNST, n=1), or malignant fibrous histiocytoma (MIH, n=1). Thus, PPARγ may be a sensitive marker for distinguishing liposarcoma from other histologic types of soft tissue sarcoma.

Transient transfection experiments were performed to characterize the activation profile of human PPARγ. To eliminate interference from endogenous receptor in the transfected cells, a chimeric PPARγ receptor was utilized that could activate transcription through a heterologous response element (see Forman et al., in Cell 53:803-812 (1995)). This chimeric protein contained the yeast GAL4 DNA binding domain linked to the ligand binding domain of human PPARγ. The GAL4-PPARγ expression vector was cotransfected into CV-1 cells with a luciferase reporter plasmid containing the GAL4 upstream activating sequence. In this assay, the thiazolidinediones BRL49653, troglitazone and pioglitazone are all seen to be effective activators of human PPARγ.

Liposarcomas have presumably acquired one or more genetic defects that interfere with the course of normal adipocytic development. The observation that PPARγ is expressed consistently in these tumors raised the possibility that the malignant cells might be forced to complete the differentiation program by maximally activating the PPARγ pathway. To address this possibility, primary cells isolated from three human liposarcomas were cultured in vitro. Primary cell strains LS857 and LS175 were derived from well differentiated liposarcomas and LS707 was derived from an intermediate grade myxoid/round cell liposarcoma (see Table 3). High grade pleomorphic liposarcoma cells could not be expanded to sufficient numbers to permit studies of differentiation. A primary leiomyosarcoma cell line LM203 was cultured as a control. To confirm that these cultures consisted of malignant tumor-derived cells, cytogenetic analysis was performed. As shown in Table 3, the karyotype of the cells in each culture was characteristic of the parent liposarcoma.

When cultured in the presence of fetal bovine serum and insulin, conditions permissive for adipocyte differentiation, all three cell lines maintain a fibroblastic morphology. LS175 cells contained small amounts of stable lipid under these conditions. When cultures were treated for 7 days with 10 μM of the PPARγ ligand pioglitazone, the cells readily accumulated lipid and adopted a morphology characteristic of mature culture adipocytes. No lipid accumulation was observed with the LM203 leiomyosarcoma cells, which do not express PPARγ. The degree of morphologically recognizable differentiation varied from 40% in the LS857 cells to 75% in the LS175 cells. After induction for 7 days with thiazolidinediones, cells maintained their differentiated morphology even when pioglitazone was withdrawn. This experiment was performed at least twice with each cell strain with quantitatively and qualitatively similar results. Induction of differentiation was also observed with the thiazolidinediones BRL49653 and troglitazone, while no effect was observed with compound 66, the inactive synthetic precursor to BRL49653.

Simultaneous exposure of incompetent cells to both PPARγ and RXR-specific ligand was investigated to determine if such combination might provide a stronger adipogenic signal than a PPARγ ligand alone. The ability of the RXR-specific ligand LG268 to promote adipocyte differentiation was investigated using NIH-3T3 fibroblasts that express PPARγ from a retroviral vector (see Tononoz et al., in Cell 79:1147-1156 (1994)). It has previously been shown that wild-type NIH-3T3 cells express RXRβ, but not PPARγ. NIH-vector and NIH-PPARγ cells were cultured as described in the Examples. At confluence, cells were treated for 7 days with no activator, 1 μM pioglitazone alone, 50 nM LG268 alone, or 5 μM thiazolidinedione and 50 nM LG268. After an additional four days of culture, cells were fixed and stained with oil red O. Data are presented in Table 1 as the range of morphologically recognizable differentiation observed for each line over three separate experiments.

### Table 1

<table>
<thead>
<tr>
<th>% lipid containing cells</th>
<th>cell line</th>
<th>no activator</th>
<th>+ pioglitazone</th>
<th>+ LG268</th>
<th>+ pioglitazone + LG268</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>NIH-PPARγ</td>
<td>2-5</td>
<td>60-70</td>
<td>50-65</td>
<td>&gt;90</td>
<td></td>
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</table>

As shown in Table 1, treatment of confluent NIH-PPARγ cells for 7 days with 50 nM LG268 resulted in significant stimulation of adipocytic differentiation, comparable to that seen with 7 days of treatment with 1 μM pioglitazone alone. Thus simultaneous exposure to both activators resulted in an additive effect. LG268 had no effect on NIH-vector cells, indicating that the adipocytic activity of this compound, like that of pioglitazone, is dependent on the presence of PPARγ. Similar results are obtained with the preadipocyte cell lines 3T3-L1 and 3T3.F442A, which express both PPARγ and RXRβ. Northern analysis confirms that pioglitazone and LG268 have an additive effect on the induction of the adipocytic-specific genes αP2 and adipin in NIH-PPARγ cells. No induction of adipocytic gene expression was observed in NIH-vector cells under similar conditions.

The ability of LG268 to promote differentiation of human liposarcoma cells was then examined. Treatment of LS857 cells for 7 days with 50 nM LG268 led to a significant degree
of adipocyte differentiation, similar to that seen with 10 μM pioglitazone. When L857 cells were treated simultaneously with LG268 and a thiazolidinedione (either pioglitazone or BRL49653), an additive effect on differentiation was observed. To further characterize the effects of PPARγ and RXR ligands on liposarcoma cells, the expression of adipocyte-specific markers were examined by Northern blotting. L857 cells, like the tumor from which they were derived, express PPARγ mRNA. Treatment of L857 cells with pioglitazone leads to the induction of two markers of terminal adipocyte differentiation, the MRNAs encoding ap2 and adipin. Simultaneous treatment with pioglitazone and LG268 results in an additive induction of adipocyte gene expression. In summary, treatment of L857 cells with thiazolidinediones and RXR-specific retinoids leads to changes in morphology and gene expression consistent with terminal adipocyte differentiation.

Terminal differentiation of white adipocytes in vitro and in vivo is characterized by permanent withdrawal from the cell cycle. A critical question is whether thiazolidinedione-induced differentiation of liposarcoma cells is accompanied by growth arrest. To address this issue, L857 cells were cultured in the presence or absence of pioglitazone. Following induction of morphologic differentiation, pioglitazone was withdrawn. After 48 hours of continued culture in the absence of pioglitazone, cells were labeled for 48 hours with bromodeoxyuridine (BrdU). Cells undergoing DNA synthesis during the labeling period should stain positive for BrdU incorporation after fixation and incubation with an enzyme-linked monoclonal antibody (see Examples). L857 cells were cultured in the presence or absence of pioglitazone. Following induction of morphologic differentiation, pioglitazone was withdrawn. After 48 hours of continued culture in the absence of pioglitazone, cells were labeled for 48 hours with bromodeoxyuridine, stained using an enzyme-linked monoclonal antibody (see Examples) and visualized microscopically. The number of cells staining positive for BrdU and/or containing visible cytoplasmic lipid is indicated in Table 2.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>#cells</th>
<th>#BrdU+</th>
<th>#lipid+ (%</th>
<th>#lipid+/BrdU+ (%</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>500</td>
<td>232(46)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>PIO/LG #1</td>
<td>530</td>
<td>173(34)</td>
<td>204(40)</td>
<td>22(4)</td>
</tr>
<tr>
<td>PIO/LG #2</td>
<td>595</td>
<td>158(26)</td>
<td>233(51)</td>
<td>17(3)</td>
</tr>
</tbody>
</table>

In the experiments shown in Table 2 (PIO/LG268#1 and PIO/LG268#2), 26–34% of the cells contained visible cytoplasmic lipid. 40–51% of the cells in this culture stained positive for BrdU incorporation by light microscopy; however, of those cells containing lipid, only 3–4% stain positive for BrdU. When differentiated cultures were trypsinized and replated, lipid-containing cells failed to reenter the cell cycle as determined by BrdU labeling. These results demonstrate the thiazolidinodione-induced differentiation of L857 cells leads to cell cycle withdrawal.

Termination differentiation of most specialized cell types, including white adipocytes, is linked to cell cycle withdrawal. Tumorigenesis is characterized by a loss of cell cycle control and a concordant block in the differentiation program. It has been demonstrated herein that most human liposarcoma cells have high levels of pioglitazone regulatory complex PPARγ/RXRs and that PPARγ and RXR-specific ligands are able to trigger terminal differentiation of primary human liposarcoma cells in vitro. These results suggest that the developmental defect in most liposarcomas is downstream of PPARγ expression, and that in at least some tumor cells this developmental block can be overcome by maximal activation of the PPARγ pathway.

While the precise nature of the developmental defects in liposarcoma is not yet clear, it is likely these defects ultimately lead to the inactivation or antagonism of one or more adipocyte transcriptional regulatory proteins. Members of both the CREB and PPAR transcription factor families have been shown to play central complementary roles in adipogenesis in murine models (see, for example, Cornellius et al., in Ann. Rev. Nutr. 14:771–774 (1994); Fonseca et al., in Curr. Opin. Genet. Dev. 5:571–576 (1995), Freytag et al., in Genes Dev. 8:1654–1663 (1994), Wu et al., in Genes Dev. 9: 2350–2363 (1995) and Yeh et al., in Genes Dev 9:168–181 (1995)). Interestingly, the CREB family has previously been implicated in the pathogenesis of human myxoid liposarcoma through the characterization of the t(12;16) translocation associated with this tumor. This rearrangement fuses the gene for the CREB family member CHOP on chromosome 12 to that of the RNA binding protein TLS on chromosome 16 (see Crozat et al., in Nature 363:640–644 (1993)). CHOP lacks a transcription activation domain and has therefore been postulated to function as a dominant negative regulator of other CREB proteins. The precise mechanism whereby TLS/CHOP contributes to differentiation arrest and tumorigenesis, however, remains to be elucidated.

The mechanisms by which differentiation is coupled to cessation of cell growth are not fully understood; however, there is mounting evidence that key proteins controlling differentiation interact directly with the cell cycle machinery. For example, the transcription factor Myc has been shown to be negatively regulated by cyclin D1-dependent kinase and to induce expression of the cyclin inhibitor p21 (see, for example, Halevy et al., in Science 267:1018–1021 (1995) and Skapek et al., in Science 267:1022–1024 (1995)).

The impact of RXR-specific activators on adipocyte differentiation has not previously been addressed. It is demonstrated herein that RXR-specific retinoids can function as adipogenic regulators through activation of the PPARγ/RXR heterodimer, and that the adipogenic activity of the heterodimer is maximal when both receptors are bound by their respective ligands. Given that PPARγ is likely to be the biologic receptor mediating the insulin-sensitizing effects of the thiazolidinediones, this observation suggests that RXR-specific ligands may also have insulin-sensitizing activity in vivo. Moreover, the insulin-sensitizing effects of thiazolidinedione ligands for PPARγ might be enhanced by simultaneous administration of an RXR-specific ligand.

The results presented herein have important implications for the pharmacologic management of liposarcoma in humans. Liposarcoma is currently managed with surgery and a judicious combination of chemotherapy and radiotherapy. Despite conventional multimodality therapy, anywhere from 25 to 75% of patients with advanced liposarcoma will die from their disease within 5 years. The present results suggest that a combination of the thiazolidinedione class of anti-diabetic drugs and RXR-specific retinoids may be useful as a non-toxic alternative to conventional chemotherapy for the treatment of disseminated or locally advanced liposarcoma. Members of the thiazolidinedione class of drugs have undergone extensive preclinical acting as anti-diabetic agents. Toglitazone is currently in phase three clinical trials in the U.S. and studies have supported its usefulness in NIDDM (see Nolan et al., in N. Engl. J. Med.)
Tumor Histology Cytogenetics Mitotic Index Cell Culture

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Histology</th>
<th>Cytogenetics</th>
<th>Mitotic Index</th>
<th>Cell Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>11S8P</td>
<td>high grade myxoid/round cell liposarcoma</td>
<td>80-91, XXXX, t(12; 16)(q13; p11) ×2</td>
<td>6.0</td>
<td>NA</td>
</tr>
<tr>
<td>11S6P</td>
<td>high grade liposarcoma, with pleomorphic, myxoid, well differentiated areas</td>
<td>ND</td>
<td>19.8</td>
<td>NA</td>
</tr>
<tr>
<td>20S8P</td>
<td>high grade liposarcoma, mixed pleomorphic and round cell</td>
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<td>7.2</td>
<td>NA</td>
</tr>
<tr>
<td>20S8P</td>
<td>well differentiated liposarcoma</td>
<td>48-50, XY, del(16)(q36), +2-4 r</td>
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<td>LS175</td>
</tr>
<tr>
<td>20S8P</td>
<td>well differentiated liposarcoma</td>
<td>48, XX, add(7)(q36), del(11)(p13), +2-2-2-2-2</td>
<td>ND</td>
<td>LS857</td>
</tr>
<tr>
<td>P144</td>
<td>well differentiated liposarcoma</td>
<td>48, XX, +2 r</td>
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<td>NA</td>
</tr>
<tr>
<td>P147</td>
<td>well differentiated liposarcoma, liposarcoma-like, sclerosing stromal</td>
<td>40-49, XX, add(9)(q34), +1-2 r, +1-2-2-2</td>
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<td>NA</td>
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<tr>
<td>P154</td>
<td>atypical liposarcoma, well differentiated liposarcoma</td>
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<td>0</td>
<td>NA</td>
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<td>P155</td>
<td>intermediate grade liposarcoma myxoid &gt; round cell component</td>
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<tr>
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<td>NA</td>
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<tr>
<td>P158</td>
<td>well differentiated liposarcoma</td>
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<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>P160</td>
<td>well differentiated liposarcoma</td>
<td>43-49, XX, add(3)(q43), -11, -13, -13, +1-3 r</td>
<td>1.1</td>
<td>NA</td>
</tr>
</tbody>
</table>

**TABLE 3-continued**

PPARγ is observed to be expressed in multiple histologic types of human liposarcoma, while PPARγ is not expressed in other human soft tissue sarcomas.

**EXAMPLE 3**

**Cell Culture**

Primary liposarcoma cells were isolated from selected freshly harvested tumors as described previously (see Sreekantiah et al., in Am. J. Pathol. 144:1121–1134 (1994) and Fletcher et al., in Am. J. Pathol. 138:515 (1991), as well as references cited therein. Primary cells were plated at a density of at least 2x10⁵ cells/ml and cultured in 60 mm dishes in RPMI containing 15% Cosmic Calf Serum (Hyclone), 20 µg/ml bovine pituitary extract (Collaborative Research) and 5 µg/ml insulin.
PPARγ- and RXR-specific ligands induce expression of markers of terminal adipocyte differentiation in PPARγ-expressing fibroblasts and human liposarcoma cells. NIH-vector, NIH-PPARγ, and LS857 cells were cultured as described above. At confluence, cells were treated for 7 days with no activator, pioglitazone alone, LG268 alone, or pioglitazone and LG268 as indicated. Total RNA (10 μg per lane) was isolated from, electrophoresed through a formaldehyde-containing agarose gel, blotted to nylon and hybridized with 32P-labeled human or murine PPARγ, human or murine αP2, and human or murine adipin cDNA using standard techniques. Equivalent amounts of intact RNA was run in each lane as indicated by ethidium bromide staining of the membrane after transfer and hybridization to a 36B4 cDNA probe.

In order to evaluate the induction of differentiation in human liposarcoma cells by thiazolidinediones and RXR-specific retinoids, LS857 cells were cultured as described above. At confluence, cells were treated for 7 days with no activator, 5 μM thiazolidinedione (BRL 49653) or pioglitazone, 50 nM LG268, or 5 μM thiazolidinedione and 50 nM LG268 as indicated. After an additional four days of culture, cells were fixed and stained with oil red O. Macroscopic view of the 60 mM culture dishes shows that thiazolidinedione alone or LG268 alone stimulate significant lipid accumulation, while simultaneous addition of both thiazolidinedione and LG268 has at least an additive effect on lipid accumulation, stimulating substantially higher levels of lipid accumulation than either agent alone.

**EXAMPLE 4**

Transfection Assays

The GAL4-hPPARγ expression vector was generously provided by V.K.K. Chatterjee. This construct contains residues 1-147 of GAL4 fused to residues 173-475 of hPPARγ and is driven by the SV40 early promoter (see Greene et al., in Gene Express. 4:281-299 (1995)). CV-1 cells were cultured in DMEM containing 10% fetal bovine serum. Transfections were performed in phenol-free DMEM containing 10% fetal bovine serum by the lipofection method using DOTAP (Boehringer Mannheim) according to the manufacturer’s instructions. After 2 hours, liposomes were removed and cells were cultured in an additional 40 hours in the presence or absence of thiazolidinediones as indicated. Luciferase and B-galactosidase assays were carried out as described previously (see Forman et al., in Cell 83:803-812 (1995)).

An SV40-GAL4-hPPARγ expression vector (100 ng/105 cells) was cotransfected into CV-1 cells with a UAS2x4 TK-LUC reporter plasmid (300 ng/104 cells) and a CMV-BGal internal control plasmid (500 ng/104 cells) as described above. Following transfection, cells were treated with indicated concentrations of Pioglitazone (Upjohn), troglitazone (Parke Davis-Warner/Lambert), BRL49653 (BIOMOL), and LG268 (Ligand Pharmaceuticals) in DMEM and applied to cells in a volume of less than 5 μl. The NIH-PPARγ and NIH-vector cells were cultured by retroviral infection as previously described (see Tontonoz et al., in Cell 79:1147-1156 (1994)) and cultured in DMEM containing 10% fetal calf serum and 5 μg/ml insulin. Differentiated cells were fixed and stained for neutral lipid with oil red O. Bromodeoxyuridine labeling was performed using the 5-bromo-2-deoxyuridine Labeling and Detection Kit II (Boehringer Mannheim) according to the manufacturer’s instructions.

These experiments demonstrate that thiazolidinediones activate PPARγ.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

That which is claimed is:

1. A method of treating liposarcomas, said method comprising administering to a subject in need of such treatment an amount of a therapeutic composition effective to ameliorate the effects of neoplastic cell proliferation of liposarcoma cells, wherein said therapeutic composition comprises at least one thiazolidinedione and at least one retinoid X receptor (RXR) selective agonist in a pharmaceutically acceptable carrier therefor.

2. A method according to claim 1 wherein said thiazolidinedione has the structure I, wherein structure I is as follows:

```
  R1
  |    |
  R2 —O— R3
     |     |
  R4 —N(R*)— R5
```

wherein:
- each of X1, X2, X3, X4, X5 and X6 is independently carbon, nitrogen or sulfur, with the proviso that at least three of the atoms forming the ring are carbon.
- R1 is hydrogen or alkyl.
- R2 is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl, arylalkenyl, substituted arylalkenyl, arylalkynyl, substituted arylalkynyl, alkyl, substituted alkyl, substituted alkyl, alkyl, alkyl, alkyl, alkyl, alkyl, alkyl, alkyl, alkyl, alkyl, alkyl, and
- R5 is substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, substituted alkyl, and
R₃ is hydrogen, hydroxy, lower alkoxy, lower alkylic, substituted lower alkylic, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl or halogen.

3. A method according to claim 2 wherein A of Formula I has the structure:

$$-Y_A-(CH₂)n-Z$$

wherein:

Y is --O-- or --S--,

n is 0 or 1,

x falls in the range of 2 up to 12; and

Z is a thiazolidinedionylo moiety.

4. A method according to claim 1 wherein the substituents of Formula I are defined as follows:

R₂ is methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy or butoxy;

R₂ is hydrogen, hydroxy or alkoxyl;

R₃ is formyl, acyl or a thiazolidinedionylo moiety;

R₄ is hydrogen; and

R₅ is hydrogen.

5. A method according to claim 2 wherein at least one of R₂, R₃, R₄ and R₅ is not hydrogen.

6. A method according to claim 2 wherein at least two of R₂, R₃, R₄ and R₅ are not hydrogen.

7. A method according to claim 6 wherein m or the sum of (m' + m'₅) with respect to the backbone of A, is less than or equal to 6.

8. A method according to claim 2 wherein:

A is --O--(CH₂)n-thiazolidinedionyl, wherein y falls in the range of 2 up to 8;

R₂ is hydrogen or lower alkyl;

R₃ is hydroxy or alkoxyl;

R₄ is formyl, acyl or a thiazolidinedionylo moiety; and

R₅ and R₆ are each hydrogen.

9. A method according to claim 1 wherein said retinoid X receptor (RXR) selective agonist is a substituted benzoic acid or derivative thereof, a substituted nicotinic acid or derivative thereof, or a substituted carboxylated furan.

10. A method according to claim 9 wherein said retinoid X receptor (RXR) selective agonist is 6-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethylv]nicotinic acid.

11. A method according to claim 9 wherein said retinoid X receptor (RXR) selective agonist is 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethenyl] benzoic acid.

12. A composition comprising at least one thiazolidinedione and at least one retinoid X receptor (RXR) selective agonist, wherein said thiazolidinedione has the structure I, wherein structure I is as follows:

$$\text{Structure I}$$

wherein:

each of X₁, X₂, X₃, X₄, X₅ and X₆ is independently carbon, nitrogen, oxygen or sulfur, with the proviso that at least three of the atoms forming the ring are carbon.

13. A composition according to claim 12 wherein A of Formula I has the structure:

$$-Y_A-(CH₂)n-Z$$

wherein:

Y is --O-- or --S--,

n is 0 or 1,

x falls in the range of 2 up to 12; and

Z is a thiazolidinedionylo moiety.

14. A composition according to claim 12 wherein the substituents of Formula I are defined as follows:

R₂ is methyl, ethyl, propyl, butyl, methoxy, ethoxy, propoxy or butoxy;

R₃ is hydrogen, hydroxy or alkoxyl;

R₄ is formyl, acyl or a thiazolidinedionylo moiety;

R₅ is hydrogen; and

R₆ is hydrogen.

15. A composition according to claim 12 wherein at least one of R₂, R₃, R₄ and R₅ is not hydrogen.

16. A composition according to claim 12 wherein at least two of R₂, R₃, R₄ and R₅ are not hydrogen.

17. A composition according to claim 16 wherein m or the sum of (m' + m'₅) with respect to the backbone of A, is less than or equal to 6.

18. A composition according to claim 12 wherein:

A is --O--(CH₂)n-thiazolidinedionyl, wherein y falls in the range of 2 up to 8;
17. R₂ is hydrogen or lower alkyl,
    R₃ is hydroxy or alkoxy,
    R₄ is formyl, acyl or a thiazolidinedionyl moiety; and
    R₅ and R₆ are each hydrogen.

19. A composition according to claim 12 wherein said
    retinoid X receptor (RXR) selective agonist is a substituted
    benzoic acid or derivative thereof, a substituted nicotinic
    acid or derivative thereof, or a substituted carboxylated
    furan.

20. A composition according to claim 19 wherein said
    retinoid X receptor (RXR) selective agonist is 6-[1-(3,5,5,
    8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)
    cyclopropyl]nicotinic acid (LG268).

21. A composition according to claim 19 wherein said
    retinoid X receptor (RXR) selective agonist is 4-[1-(3,5,5,
    8,8-pentamethyl-5,6,7,8-tetrahydro-naphthalen-2-yl)ethenyl]
    benzoic acid.

* * * * *