Resveratrol, a stilbenoid antioxidant found in grapes, wine, peanuts and other berries, has been reported to have hypolipidemic properties. We investigated whether resveratrol and its three analogs (pterostilbene, piceatannol and resveratrol trimethyl ether) would activate the peroxisome proliferator-activated receptor alpha (PPARα) isoform. This nuclear receptor is proposed to mediate the activity of lipid-lowering drugs such as the fibrates. The four stilbenes were evaluated along with ciprofibrate (positive control) at 1, 10, 100, 300 μM concentrations, for the activation of endogenous PPARα in H4IIEC3 cells. Cells were transfected with a peroxisome proliferator response element-AB (rat fatty acyl CoA β-oxidase response element)—luciferase gene reporter construct. Of the four analogs, pterostilbene demonstrated the highest induction of PPARα at 9- and 9-14 fold increases in luciferase activity at 100 and 300 μM, respectively, relative to control. The maximal luciferase activity responses to pterostilbene at 100 μM are similar to those obtained with the hypolipidemic drug, ciprofibrate. These results suggest that pterostilbene acts as a PPARα agonist, like that of the fibrate class, and may be a more effective hypolipidemic agent than resveratrol.
Fig. 2
PTEROSTILBENE AS A NEW AGONIST FOR THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA ISOFORM

REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 60/602,784, filed Aug. 19, 2004, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] This invention relates to pterostilbene, an analog of resveratrol, found in grapes, wine, peanuts and other berries. Pterostilbene activates the peroxisome proliferator-activated receptor alpha (PPARα) isofrom, a receptor proposed to mediate the activity of lipid-lowering drugs. As a PPARα agonist, pterostilbene is capable of acting as an effective hypolipidemic agent.

[0004] 2. Description of the Relevant Art


[0007] Pterostilbene is another grape compound that also was found to have antioxidant (Rimando et al. 2002. J. Agric. Food Chem. 50: 3453-3457; Stivala et al., supra) and cancer chemopreventive property similar to resveratrol (Rimando et al., supra). Pterostilbene has antidiabetic (Mandlik et al. 1997. J. Nat. Prod. 60: 609-610) properties, and inhibits the enzymes cyclooxgenase-1 (COX-1) and COX-2, interfering anti-inflammatory properties (Likhitwilayasuwat et al. 2002. Planta Medica 68: 841-843). Furthermore, pterostilbene is cytotoxic to a number of cancer cell lines in vitro (Rimando et al., 1994. Nat. Prod. Lett. 4: 267-272).


[0009] Thus, in view of reports on the hypolipidemic property of resveratrol, the three analogs were of interest because their biological activity profiles are similar to that of resveratrol and in some assays are reported to be more potent than resveratrol. The goal of this work was to investigate whether these analogs are PPARα agonists.

SUMMARY OF THE INVENTION

[0010] We have investigated the property of pterostilbene as an agonist of PPARα and have determined that pterostilbene can be used as a hypolipidemic agent.

[0011] In accordance with this discovery, it is an object of the invention to provide a pharmaceutical composition that acts as a hypolipidemic agent.
It is a further object of the invention to provide a pharmaceutical composition that specifically activates PPARα.

In particular, this invention provides a method of treating dyslipidemias in individuals at risk of cardiovascular disease by administering a pharmaceutical composition containing pterostilbene.

It is further part of this invention to provide a method of lowering cholesterol levels in individuals by administering a pharmaceutical composition containing pterostilbene.

Also part of this invention is a kit, comprising a pharmaceutical composition containing pterostilbene; and instructions for the use of the kit.

Other objects and advantages of this invention will become readily apparent from the ensuing description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the structures and formulae of resveratrol, pterostilbene, piceatannol, resveratrol trimethyl ether, and ciprolibrate.

FIG. 2 shows the effect of stilbenes on PPARα in H4IEC3 cells transfected with the PPRE-AB-luciferase reporter gene plasmid. Cip, ciprolibrate; Res, resveratrol; Pte, pterostilbene; Pt, piceatannol; Rte, resveratrol trimethyl ether; ns, not significantly different from control, p=0.05; **, significantly different from control, p=0.001; ***, highly significantly different from control, p=0.0001; n=4.

DETAILED DESCRIPTION OF THE INVENTION

Dyslipidemias are disorders of lipoprotein metabolism, including lipoprotein overproduction or deficiency. These disorders may be manifested by elevation of the serum total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride concentrations, and a decrease in the high-density lipoprotein (HDL) cholesterol concentration. Resveratrol has been shown to have hypolipidemic properties in rat feeding studies causing lowering of triglyceride (Miura et al., supra) and serum cholesterol levels (Miura et al., supra; Kollar et al. 2000. Vniirn Lekarsvi 46: 856-860). On the contrary, a study in rabbits showed no difference in lipoprotein-cholesterol concentration between the control group and the group that received oral resveratrol (Wilson et al. 1996. Life Sci. 59: 15-21). Additionally, resveratrol promoted atherosclerotic development in these animals. On the basis of results of studies on the effect of resveratrol in human aortic cells, it was hypothesized that it may confer cardiovascular protection by functioning as a pleiotropic cellular effector (Wu et al., 2004. In: Phytochemicals: Mechanisms of Action, Meskin, M. S., ed., CRC Press I.C., Boca Raton, Fla., pages 145-161). Results from studies in Donju rats showed the hypocholesterolemic activity of resveratrol to be due to increased excretion of neutral sterols and bile acids in the feces (Miura et al., supra).

“PPAR ligands or agonists” evolved as a group of structurally diverse compounds that activate these transcription factors, and emerged as an important class of therapeutic agents as PPARs have become an important molecular target to treat human metabolic disorders. PPARs play a central role in lipid homeostasis in regulating fatty acid metabolism and plasma lipoproteins. The fibrate drugs, such as ciprolibrate that was used in this study, were found to be ligands for PPARα and their activation of PPARα provided a mechanistic explanation for their clinical efficacy in treating cardiovascular diseases (Roberts and Moffat. 2001. Comments on Toxicology 7: 259-273).

Three analogs of resveratrol: pterostilbene, piceatannol and resveratrol trimethyl ether have been shown to exhibit some of the same biological activities as resveratrol; however, these analogs also have individual activities which they do not share with resveratrol. The capability of resveratrol and the analogs: pterostilbene, piceatannol and resveratrol trimethyl ether to affect PPARs, in particular, PPARα, and to thus affect lipid metabolism, was examined.

The finding that pterostilbene is an agonist for PPARα and that it possesses an activity comparable to a clinically prescribed hypolipidemic fibrate drug, provides a possible natural source alternative for the treatment of dyslipidemias. Whether or not resveratrol is a cholesterol lowering agent, the mechanism by which it does so does not appear to be via activation of PPARα, as is shown in this study. Results from this study suggest that pterostilbene may be a more effective hypolipidemic agent with differing mechanisms of lipid lowering action than that of resveratrol.


EXAMPLES

Having now generally described this invention, the same will be better understood by reference to certain specific examples, which are included herein only to further illustrate the invention and are not intended to limit the scope of the invention as defined by the claims.

Example 1

Analogs, Cell Lines, and Reagents

Resveratrol and piceatannol were commercial samples obtained from Sigma-Aldrich (St. Louis, Mo.) and Calbiochem (San Diego, Calif.), respectively. H4IEC3 cells were obtained from the American Type Culture Collection (Rockville, Md.). The PPRE-AB luciferase gene reporter construct was obtained from Dr. Daniel J. Noonan (Department of Biochemistry, University of Kentucky, Lexington, Ky.). The luciferase assay kit was obtained from Promega Corporation (Madison, Wis.). NMR experiments were carried out on a Bruker Avance DRX (500 MHz) instrument. PTLC, Merck Si gel F254 20x20 cm, 0.5 mm thick plate (WVR Scientific, Atlanta, Ga.). All solvents used were HPLC grade (Fisher Scientific, Suwanee, Ga.).

Pterostilbene and resveratrol trimethyl ether were prepared by partial methylation of resveratrol. To a solution of resveratrol (150 mg in 3.0 ml of MeOH) diazomethane was added dropwise, and the reaction was monitored by thin layer chromatography (TLC) for the methylated products.
The reaction solution was dried under vacuum. The partially methylated products were purified by preparative TLC (developing solvent, hexane: ETOAc, 8:2; RF 0.6 and 0.8 for pterostilbene and resveratrol trimethyl ether, respectively). The identity and structure of these compounds were confirmed by comparison with published spectroscopic data.

Example 2

Activation of PPARα in Rat Cells

[0027] Conditions for activation were essentially as described in Jaradat et al. (2002. Planta Medica 68: 667-671). Briefly, H4IEC3 cells, a rat hepatoma cell line, were grown in a 150 mm Petri dish containing Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum. Upon reaching 75% confluency, cells were transfected with PPRE-AB (peroxisome proliferator response element with rat fatty acyl CoA β-oxidase AB promoter region sequence)—luciferase gene reporter construct, by electroporation (Square electroporator model T820) at 190V for 70 sec and 1 pulse. Transfected cells along with the medium were plated in 96 well microtiter plates, at 100,000 cells/well. After 24 hr incubation, ciprofibrate at 10 and 100 μM, resveratrol and its three analogs at 1, 10, 100, and 300 μM, were added to the medium containing cells. FIG. 1 shows the structures and formulae of resveratrol, pterostilbene, piceatannol, resveratrol trimethyl ether, and ciprofibrate. After incubation with the test compounds for 30 hr, the cells were lysed and the luciferase activity of the supernatant was determined using a luminometer (Packard Instrument Company, Meriden, Conn.). Data were analyzed using GraphPad-Prism software.

[0028] Of the four stilbene analogs, pterostilbene demonstrated the highest induction of luciferase activity at 100 and 300 μM (FIG. 1). The maximal luciferase responses to pterostilbene at 100 and 300 μM were about 7 fold and 14 fold, respectively, when compared to the corresponding control values (n=4) in H4IEC3 cells. The maximal luciferase activity responses to ciprofibrate at 100 μM was about 5 fold compared to control (n=4). These results showed that PPARα activation by pterostilbene (32932788 relative luciferase units) is comparable to that of ciprofibrate (19460±1466 relative luciferase units) at 100 μM. Piceatinol and resveratrol trimethyl ether induced luciferase activity only slightly at all the concentrations tested. At 1 and 10 μM, pterostilbene, as well as resveratrol, only minimally induced luciferase activity. Resveratrol was toxic to H4IEC3 cells at 100 and 300 μM. These results indicate that pterostilbene, like ciprofibrate, acts as an agonist of PPARα in H4IEC3 cells, whereas the remaining stilbene analogs are not activators of PPARα.

Example 3

Effect of Pterostilbene and Resveratrol in In Vivo Hamster Studies

[0029] Male golden Syrian hamsters (Charles River, Wilmington, Mass.), initial weight ranging from 34-41 g, were fed a powdered stock diet (Rodent Lab Chow 5001, Purina Mills, St. Louis, Mo.) for 7 days. The animals were placed in individual wire-bottom cages in a room kept at 20-22°C, 60% rh and 12 h light and dark cycle. Following the initial 7 day period, 8-10 animals were randomly assigned from a weight sorted list to each of five test diets (α-cellulose control and test substance). Food intake was measured twice each week and body weights were monitored once a week. After 21 days on the treatment diets, the animals were killed, blood was collected and analyzed for total cholesterol by the cholesterol oxidase method and lipoprotein cholesterol, by size exclusion chromatography, as previously described (German et al. 1996. Nutrition Res. 16: 1239-1249). All animal procedures were approved by the Animal Care and Use Committee, Western Regional Research Center, USDA, Albany, Calif. and conformed to the principles in “Guide for the Care and Use of Laboratory Animals” (Committee on Care and Use of Laboratory Animals 1985). Plasma blood glucose was determined using a blood glucose meter (Fast Glucose Meter-Precision Xtra, Medisense, Bedford, Mass., USA).

[0030] Diets were prepared by dissolving powdered cholesterol into warmed butterfat, followed by the addition of corn and fish oil. The small amounts of test ingredients were dissolved in about 50 ml of ethanol and added to the stirring dry ingredients. The liquid fat was also added to the dry ingredients while stirring. The composition is shown in Table 1.

### TABLE 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butterfat, anhydrous</td>
<td>80</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>100</td>
</tr>
<tr>
<td>Fish Oil, Menhaden</td>
<td>20</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.5</td>
</tr>
<tr>
<td>Cellulose, microcrystalline</td>
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</tr>
<tr>
<td>Casein</td>
<td>200</td>
</tr>
<tr>
<td>Starch, corn</td>
<td>497.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>3</td>
</tr>
<tr>
<td>Mineral Mix</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin Mix</td>
<td>10</td>
</tr>
<tr>
<td>Test Substance</td>
<td>25 mg/kg</td>
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</table>

[0031] Compared to the control group, plasma low density lipoprotein (LDL) cholesterol was 29% lower in the pterostilbene-fed group and 20% lower in the resveratrol-fed group, at 25 mg/kg diet (Table 2). Total plasma cholesterol was 18% lower with pterostilbene and 14% lower with resveratrol. LDL/HDL ratio was statistically significantly lower for pterostilbene but not for resveratrol, compared to control, at this diet concentration. Weight gain and HDL were similar for all groups. Compared to the control group, the plasma glucose level of only the pterostilbene-fed group was lowered.

### TABLE 2

| Effect of Pterostilbene and Resveratrol on Plasma Lipoprotein Cholesterol Levels in Hamsters |
|-----------------------------------------------|----------------|----------------|----------------|
|                                                | Control         | Pterostilbene* | Resveratrol*   |
|                                                | AVG  | SEM  | AVG  | SEM  | AVG  | SEM  |
| Plasma Lipoprotein Cholesterol (mg/dL)         |      |      |      |      |      |      |
| VLDL                                           | 99.3 | 15.3 | 82.7 | 15.7 | 83.6 | 13.0 |
| LDL                                            | 320.9| 4.9  | 228.1| 4.2  | 257.4| 8.0  |
TABLE 2-continued

<table>
<thead>
<tr>
<th>Effect of Pterostilbene and Resveratrol on Plasma Lipoprotein Cholesterol Levels in Hamsters</th>
<th>Control</th>
<th>Pterostilbene*</th>
<th>Resveratrol*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AVG</td>
<td>SEM</td>
<td>AVG</td>
</tr>
<tr>
<td>HDL</td>
<td>127.4</td>
<td>1.1</td>
<td>137.0</td>
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<tr>
<td>Total</td>
<td>547.6</td>
<td>6.7</td>
<td>447.8</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>2.6</td>
<td>0.37</td>
<td>1.8</td>
</tr>
<tr>
<td>Final Weights (g)</td>
<td></td>
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<td>0.39</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>0.62</td>
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</table>

*Concentration: 25 ppm

[0032] In addition, at the same time the feeding experiments with pterostilbene and resveratrol were done, two tomato and five eggplant varieties were also tested. Freeze-dried plant materials were fed at about 10% of total diet. Test substances (pterostilbene and resveratrol) were found to be more effective than the plant materials for lowering the plasma cholesterol levels (data not shown).

[0033] Differences between treatments were tested by two-tailed t-test assuming equal variance, and were considered significant at p<0.05.

[0034] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

[0035] It is understood that the foregoing detailed description is given merely by way of illustration and that modifications and variations may be made therein without departing from the spirit and scope of the invention.

We claim:

1. A pharmaceutical composition comprising a therapeutically effective amount of pterostilbene and a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1 wherein the therapeutically effective amount of pterostilbene is an amount sufficient to lower lipid levels in an individual.

3. A nutraceutical composition comprising an amount of pterostilbene sufficient to lower lipid levels in an individual and a nutraceutically acceptable carrier.

4. The nutraceutical composition of claim 3 wherein the composition comprises extracts of grapes or berries of *Vaccinium* and a nutraceutically acceptable carrier.

5. A method of lowering lipid levels in an individual comprising administering to an individual a therapeutically effective amount of the composition of claim 1.

6. A method of treating dyslipidemias in individuals at risk of cardiovascular disease by administering to an individual a therapeutically effective amount of the composition of claim 1.

7. A method of lowering lipid levels in an individual comprising administering to an individual an effective amount of the composition of claim 3 or claim 4.

8. A kit for the treatment of dyslipidemias, wherein the kit comprises:

   a composition comprising an effective amount of pterostilbene according to claim 1;

   and a container housing the composition.

9. A kit for lowering lipid levels in an individual, wherein the kit comprises:

   a composition according claim 3 or claim 4; and a container housing the composition.

* * * * *