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Title: THE USE OF PAN-PPAR AGONISTS FOR PREVENTION AND TREATMENT OF HUNTINGTON’S DISEASE AND TAUOPATHIES

Abstract: A method of prevention and treatment for a neurodegenerative disease with a pan-PPAR agonist, such as bezafibrate. In particular, the present invention provides that pan-PPAR agonists enhance PPAR related responses in both the central nervous system and peripheral tissues in Huntington’s disease (HD) and tauopathy. Therapeutic compositions comprising one or more pan-PPAR agonist(s), and kit thereof, for preventing and treating a neurodegenerative disease or disorder are also provided.
THE USE OF PAN-PPAR AGONISTS FOR PREVENTION AND TREATMENT
OF HUNTINGTON'S DISEASE AND TAUOPATHIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 61/557,729, filed November 9, 2011, the entire contents of which are incorporated by reference herewith.

GOVERNMENT FUNDING

[0002] This invention was made with Government support under Grant Numbers P01AG14930 and AG014930 awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that act as ligand-dependent transcription factors (Kersten et al 2000). PPARs are also ligand-inducible transcription factors belonging to the steroid, thyroid and retinoid receptor superfamily and also termed nuclear hormone receptors (Desvergne and Wahli 1999; Straus and Glass 2001). Nuclear receptors directly bind to DNA and regulate gene expression through transcriptional co-activation (Nolte et al. 1998; Berger and Moller 2002; Castrillo and Tontonoz 2004). The PPAR subfamily is comprised of three isoforms: PPAR-a, PPAR-β/δ and PPAR-γ, and these isoforms share structural homology in various species (Desvergne and Wahli 1999; Bishop-Bailey 2000; Buchan and Hassall 2000; Straus and Glass 2001). PPARa, β, and γ are the three commonly known PPAR isoatypes. PPARa is predominantly expressed in the liver, kidney, muscle, adipose, and heart, whereas PPARβ is found in the brain, adipose, and skin, and PPARγ is expressed ubiquitously (Bensinger and Tontonoz 2008). These transcription factors have been linked to lipid transport, metabolism, and inflammation pathways (Bensinger and Tontonoz 2008). Because of this, synthetic PPAR agonists have been generated as therapeutic agents for the treatment of diabetes and metabolic diseases (Schulman 2010; Wang 2010).
PPAR are activated by small, lipophilic compounds and form heterodimers with the retinoid X receptor-a (RXR) in the cytoplasm for full activation (van Neerven and Mey 2007). After activation the PPAR/RXR heterodimer binds to the specific DNA sequence (peroxisome proliferator response element; PPRE) on the promoter region of PPAR target genes (Desvergne and Wahli 1999; Qi et al. 2000) to modulate transcriptional activity. Specific binding of PPAR on DNA sequences leads to activation of hundreds of gene cascades involved in several biological processes (Qi et al. 2000). In the absence of ligands, PPAR and RXR heterodimers bind to co-repressor complexes and suppress gene transcription (Ziouzenkova and Plutzky 2008).

While binding of PPAR with specific ligands leads to release of corepressors from heterodimers and recruitment of co-activators, followed by activation of the basal transcriptional machinery (Kamei et al. 1996; Desvergne and Wahli 1999; Straus and Glass 2001), dietary lipids and their metabolites, fatty acids and eicosanoids are the natural ligands for PPAR. However, these receptors are also activated by synthetic ligands such as thiazolidinediones, fibrates, W501516 and L-165041 (Desvergne and Wahli 1999; Straus and Glass 2007). Several non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, fenoprofen and indomethacin also activate PPAR-a and PPAR-γ (Lehmann et al. 1997). It was postulated that the anti-inflammatory actions of these drugs may arise from their ability to bind to PPAR, and subsequent activation of these receptors (Jiang et al. 1998; Ricote et al. 1998; Casper et al. 2000; Heneka and Landreth 2007).

Different PPAR isoforms develop from a common PPAR gene and show tissue dependent patterns of expression during fetal development and are involved in differentiation of adipose tissue, brain, skin, liver, muscle and placenta (Desvergne and Wahli 1999; Gofflot et al. 2007). On the basis of target genes and differential localization in the tissues, these isoforms perform different pharmacological, physiological and biological functions and exhibit different ligand specificities (Desvergne and Wahli 1999). PPAR-a is activated by natural fatty acids and synthetic fibrate ligands and regulates metabolism of lipid and apolipoproteins. PPAR-γ is involved in regulation of adipocyte differentiation (adipogenesis), glucose metabolism, insulin sensitivity and cell growth and is activated by natural ligands as well as synthetic glitazone ligands, while, PPAR-β/δ regulates lipid and glucose metabolism.
PPARs can affect metabolism and inflammation in the central nervous system (Heneka and Landreth 2007), suggesting that they can play a role in the pathogenesis of neurodegenerative diseases. PPAR agonists increase oxidative phosphorylation capacity in mouse and human cells (Bastin et al 2008; Hondares et al 2006; Wenz et al 2010), and enhance mitochondrial biogenesis.

Prior reports have demonstrated beneficial effects of PPARy agonists, such as thiazolidinediones (TZD, also called glitazones) (Kaundal and Sharma 2010), in models of stroke (Culman et al 2007) and Alzheimer's disease (Heneka et al 2005; Jiang et al 2008; Nicolakakis et al 2008; Nicolakakis and Hamel 2010). Fibrates, such as fenofibrate (Rakhshandehroo 2010), are another class of PPAR agonists (Abourbih et al 2009; Munigoti and Rees 2011; Staels et al 2008) that primarily target the PPARa pathway. Like TZD, fenofibrate has demonstrated promising protective effects in models of neurodegenerative diseases, including Parkinson's disease (Kreisler et al 2010), and brain injury (Chen et al 2007). Interestingly, the neuroprotective effects of PPAR agonists seem to occur through a common mechanism involving the reduction of oxidative stress and inflammation (Heneka et al 2005; Jiang et al 2008; Nicolakakis et al 2008; Nicolakakis and Hamel 2010; Chen et al 2007).

Bezafibrate is a member of the fibrate family that predominantly activates PPARa, but can also act on PPARβ and γ (Tenenbaum et al 2005). It can therefore be considered a pan-PPAR agonist. Recently, the administration of bezafibrate was shown to increase PGC-1α expression, mitochondrial DNA and ATP levels; and to increase life span and delay myopathy in a COX-10 subunit deficient mouse model of mitochondrial myopathy (Wenz et al 2008). Bezafibrate enhances lipid metabolism and oxidative capacity (Tenenbaum et al 2005; Bastin et al 2008). Bezafibrate is an effective cholesterol lowering drug which is used to lower cholesterol and triglycerides and increase high density lipoprotein (HDL).

Huntington's disease (HD), is a fatal, dominantly inherited progressive neurodegenerative disease, caused by an abnormal CAG repeat expansion in the huntingtin ( htt) gene. The disease is characterized by progressive motor impairment, personality changes, psychiatric illness and gradual intellectual decline, leading to death 15-20 years after onset (Vonsattel and DiFiglia 1998). Neuropathological analysis shows a preferential and progressive loss of the medium spiny neurons (MSNs) in the striatum,
although cortical atrophy and degeneration of other brain regions occur in later stages of the disease (Vonsattel and DiFiglia 1998; Hayden and Kremer 2001; Zuccato et al 2010). Several transgenic mouse models exist that recapitulate the main features of HD, and which have been used for development and testing of new therapeutic interventions. Transgenic mouse models either contain htt N-terminal fragments, usually the first 1 or 2 exons of the human htt gene with the CAG expansion, or the full-length human HD gene with an expanded CAG tract. All these models share features with human HD. The most extensively studied are the R6/2 mice, which express exon-1 of the human htt gene, and which initially show behavioral and motor deficits at 6 weeks after birth. Subsequently, the phenotype of the R6/2 mice develops rapidly manifesting tremor, clasping, weight loss, diabetes, behavioral abnormalities, and reduced life span of 10-13 weeks (Mangiarini et al 1996; Menalled and Chesselet 2002).

Transcriptional dysregulation, protein aggregation, mitochondrial dysfunction and enhanced oxidative stress have been implicated in the disease pathogenesis. A critical role of peroxisome proliferator activated receptor (PPAR)-γ-coactivator 1α (PGC-1α), a transcriptional master co-regulator of mitochondrial biogenesis, metabolism and anti-oxidant defenses, has been identified in HD. Interest in the role of PGC-1α in HD pathogenesis initially came from studies of PGC-1α knockout mice (PGC-1α KO), that display neurodegeneration in the striatum, which is also the brain region most affected in HD (Lin et al. 2004; Leone et al. 2005). PGC-1α also plays a role in the suppression of oxidative stress, and it induces mitochondrial uncoupling proteins and antioxidant enzymes, including copper/zinc superoxide dismutase (SOD1), manganese SOD (SOD2), and glutathione peroxidase (Gpx-1) (St-Pierre et al 2006). Oxidative damage is a well characterized feature which is documented in plasma of HD patients, HD postmortem brain tissue, and in HD transgenic mice (Browne and Beal 2006; Hersch et al. 2006).

Using striata from human HD patients, striata from HD knock-in mice and the STHdhQ11 cell-based HD model, Cui et al. (Cui et al. 2006) showed marked reductions in mRNA expression of PGC-1α, and interference of mutant htt with the CREB/TAF4 complex was shown to be instrumental in this reduction. Down-regulation of PGC-1α significantly worsened the behavioral and neuropathological abnormalities in a PGC-1α knock-out HD knock-in mouse model (PGC-1α KO/KI). Administration of a
lentiviral vector expressing PGC-la into the striatum of R6/2 mice, was neuroprotective in that it increased the mean neuronal volume of medium spiny neurons (Cui et al. 2006). Caudate nucleus microarray expression data from human HD patients showed significant reductions in 24 out of 26 PGC-la target genes (Weydt et al. 2006). These authors also found reduced PGC-la mRNA expression in striata of the N171-82Q transgenic mouse model of HD.

[0013] Subsequent studies were carried out, which showed that the ability to upregulate PGC-la in response to an energetic stress, produced by administration of the creatine analogue, guanidinopropionic acid, was markedly impaired in HD transgenic mice (Chaturvedi et al. 2009; Chaturvedi et al. 2010). PGC-la plays a critical role in mitochondrial biogenesis in muscle, and in influencing whether muscle contains slow-twitch oxidative or fast-twitch glycolytic fibers (Lin et al. 2002). Impaired generation of ATP in muscle and a myopathy occurs in gene-positive individuals at risk for HD, HD patients and HD transgenic mice (Gizatullina et al. 2006; Kosinski et al. 2007; Turner et al 2007). Impaired PGC-la activity was observed in muscle of HD transgenic mice, and in myoblasts and muscle biopsies from HD patients (Chaturvedi et al 2010). A pathologic grade-dependent significant reduction in numbers of mitochondria in striatal spiny neurons, which correlated with reductions in PGC-la and the mitochondrial transcription factor a (Tfam) was also showed (Kim et al 2010). Sequence variation in the PGC-la gene modifies the age of onset of HD (Weydt et al 2009; Taherzadeh-Fard et al 2009). Stimulation of extra-synaptic NMDA receptors, which is detrimental, impairs the PGC-la cascade in HD mice (Okamoto et al 2009). Impaired PGC-la was shown to correlate with lipid accumulation in brown adipose tissue of HD transgenic mice (Phan et al 2009). These findings in concert, strongly implicate reduced expression of PGC-la in HD pathogenesis. If impaired PGC-la transcriptional activity is playing an important role in HD pathogenesis, then pharmacologic agents such as bezafibrate which increase its levels and activity might be beneficial.

[0014] With respect to tauopathies, although previous reports have shown that PPAR agonists can reduce amyloid-β (Aβ), studies to clarify the role of PPARs in Alzheimer's disease are necessary. In particular, the relationship between PPARs and the protein tau should be explored. Presently, whether a pan-PPAR agonist can be beneficial
in models of Alzheimer's disease and tauopathy, specifically, the effect of bezafibrate administration in P301S mice was investigated.

[0015] Increased phosphorylation and accumulation of tau within neurons are important hallmarks of Alzheimer's disease and tauopathies. P301S transgenic mice, which carry the mutated human tau gene (P301S mutation), develop progressive tau pathology, behavioral (Scattoni et al 2010) and synaptic deficits (Yoshiyama et al 2007), and microglial activation (Yoshiyama et al 2007; Bellucci et al 2004).

[0016] There is a large body of evidence demonstrating the importance of PPARs in lipid metabolism, energy metabolism, and inflammation. Several groups have investigated the role of PPARs in the central nervous system and their effects in models of neurodegeneration (Heneka and Landreth 2007).

[0017] PPARy agonists such as pioglitazones and rosiglitazones have been widely used in models of neurodegenerative diseases. Previously, it was reported that administration of pioglitazone extended survival, and attenuated neuronal loss, gliosis, and oxidative stress in a mouse model of amyotrophic lateral sclerosis (ALS) (Kiaei et al 2005). Similar results were found in the transgenic mouse models of Alzheimer's disease (AD) (Landreth 2007). These drugs had protective effects in transgenic mice modeling AD by reducing Aβ levels, inflammation, (Heneka et al 2005) and cerebrovascular dysfunction (Nicolakakis et al 2008). In addition to behavioral improvement (Escribano et al 2009), rosiglitazone also enhanced mitochondrial biogenesis (Strum et al 2007). Upregulation of PPARy in neuroblastoma cells transfected with the human amyloid precursor gene (APP) was neuroprotective as evidenced by a reduction of H$_2$C>2-induced cell death and Aβ secretion (d' Abramo et al 2005). Other PPAR agonists have been tested as potential therapeutic agents for the treatment of neurodegenerative diseases. PPARa agonists, such as fenofibrate, show promising effects in mouse models of Parkinson's disease (PD) (Kreisler et al 2010) and brain injury (Besson et al 2005; Deplanque et al. 2003). In the latter, data showed that the neuroprotection was due to elevated antioxidant enzyme activities and reduced markers of inflammation. In primary neuronal cells, administration of Wy-14,463, a PPARa agonist, reduced Aβ-induced cell death, reactive oxygen species (ROS) production, and elevated calcium level by upregulating mitochondrial antioxidant enzymes (Santos et al 2005).
Tauopathies are a class of neurodegenerative diseases or effects of CNS trauma characterized by a pathological aggregation of tau protein in the human brain. The best known of these illnesses is Alzheimer's disease (AD), where tau protein is deposited within neurons in the form of neurofibrillary tangles (NFTs). Tangles are formed by hyperphosphorylation of a microtubule-associated protein known as tau, causing it to aggregate in an insoluble form (These aggregations of hyperphosphorylated tau protein are also referred to as PHF, or "paired helical filaments"). Other tauopathies include: Progressive supranuclear palsy; Dementia pugilistica (chronic traumatic encephalopathy); traumatic encephalopathy; Frontotemporal dementia and parkinsonism linked to chromosome 17; Lytico-Bodig disease (Parkinson-dementia complex of Guam); Tangle-predominant dementia; Ganglioglioma; gangliocytoma; Meningioangiomatosis; Subacute sclerosing panencephalitis; lead encephalopathy; tuberous sclerosis; Hallervorden-Spatz disease; lipofuscinosis; Pick's disease; corticobasal degeneration; Argyrophilic grain disease (AGD); corticobasal degeneration; Frontotemporal dementia; and Frontotemporal lobar degeneration. The non-Alzheimer's tauopathies are grouped together as members of "Pick's complex". For the purposes of this patent application, Parkinson's disease is not a tauopathy.

Although a number of studies have shown that agonists targeting individual PPARs have neuroprotective efficacy, there have not previously been studies of pan-PPAR agonists in transgenic mouse models of neurodegenerative diseases. Other approaches to achieving pan-PPAR effects include utilizing combinations of agonists that act either at the individual PPAR subtypes or at two PPAR subtypes. Examples of the latter include the glitazars, which operate as agonists of PPAR α and γ, and include aleglitazar, mura(glitazar and tesaglitazar (Staels 2002). PPAR gamma agonists include the thiazolidinediones, also known as glitazones, which include rosiglitazone, pioglitazone, and troglitazone which are marketed drugs, as well as experimental agents MCC-555, rivoglitazone, and ciglitazone. NSAIDS such as ibuprofen and naproxen activate PPARγ (Dill et al 2010). Other PPARγ agonists include GW1929, azelacyl PAF, and BUT.13. PPAR alpha agonists include fibrates other than bezafibrate, such as CP-751461, CP868388, GW7647 and WY-14643. PPAR-beta/delta agonists include GW0742 and L165.041.
There is still a need to develop an effective potent therapeutic method for the treatment of neurodegenerative diseases, particularly for Huntington's disease and/or tauopathies, since there is as yet no cure for these disorders, and no therapy to delay the onset of symptoms.

SUMMARY OF THE INVENTION

The present invention provides a method of preventing and treating a neurodegenerative disease or disorder by administering to a subject in need a therapeutic amount of a composition comprising a pan-PPAR agonist, including wherein the pan-PPAR agonist is bezafibrate or an active derivative thereof. In certain embodiments, the neurodegenerative disease is Huntington's disease or a tauopathy. The invention provides that bezafibrate is beneficial in Huntington's disease (HD) and in tauopathies. In HD, administration of bezafibrate provides significant improvements in behavior and survival, and prevents negative biochemical changes as well as neural and muscular degeneration. In tauopathy, treatment with bezafibrate reduces behavioral hyperactivity and disinhibition and reduces tau hyperphosphorylation, neurofibrillary tangle formation, inflammation, oxidative stress in spinal cord, and improved pathology in the brown adipose tissue.

The tauopathy may be a member of Pick's complex (aka, Rick's disease and/or Rick's dementia), which typically affects the frontal and/or anterolateral temporal lobes (also classified under the term frontotemporal dementia (FTD)). The member of Pick's complex/disease includes, but is not limited to, Progressive supranuclear palsy; Dementia pugilistica (chronic traumatic encephalopathy); traumatic encephalopathy; Frontotemporal dementia and parkinsonism linked to chromosome 17; Lytico-Bodig disease (Parkinson-dementia complex of Guam); Tangle-predominant dementia; Ganglioglioma; gangliocytoma; Meningioangiomatosis; Subacute sclerosing panencephalitis; lead encephalopathy; tuberous sclerosis; Hallervorden-Spatz disease; lipofuscinosis; Pick's disease; corticobasal degeneration; Argyrophilic grain disease (AGD); corticobasal degeneration; Frontotemporal dementia; or Frontotemporal lobar degeneration.

The present invention further provides a composition, and a kit containing the composition, for preventing and treating a neurodegenerative disease or disorder comprising an effective amount of a pan-PPAR agonist. In certain embodiments, the pan-
PPAR agonist is bezafibrate. In certain embodiments, the composition further comprises a pharmaceutically suitable and acceptable carrier or adjuvant mixed with, and/or in conjunction with the pan-PPAR agonist. The composition of the present invention can be formulated in any pharmaceutically suitable and/or acceptable formulations for any suitable and/or acceptable administration routes, including, but not limited to, oral tablet or capsule, parental injectable solution or suspension, or subcutaneous patches. The composition comprising a pan-PPAR agonist can further be formulated to a suitable controlled-release, sustained-release, and/or delayed-release formulation. The composition of the present invention can be administered alone, or in combination with any suitable agent or compound to enhance the effect for prevention and treatment of the neurodegenerative disease, and/or reduce any syndroms directed or indirecd associtated with the disease and/or side effects resulted from the treatment.

The present invention further provides a method for preventing and treating a neurodegenerative disease utilizing a combination of agonists that together target all PPARs to achieve enhanced pan-PPAR neuroprotective effects, as evidenced in transgenic mouse models of human neurodegenerative diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Bezafibrate restores the PGC-1α signaling pathway in R6/2 mice. Figure 1(A) illustrates a relative expression of full-length (FL) and N-truncated (NT) isoforms of PGC-1α, PPARα, γ, δ, and the downstream target genes, nuclear respiratory factor NRF-1, Cyt c, Tfam, ATP synthase, as well as the oxidative stress response genes HO-1, Nrf-2 and Gpx-1 in brain of R6/2 mice on a standard diet or on the bezafibrate diet. The levels of each gene transcript were normalized to that of β-actin and expressed as fold variation relative to the wild-type mice on a standard diet. The asterisks and symbols represent the significance levels calculated by unpaired, Student's two-tailed t test: *p < 0.05 compared to the wild-type controls; §p < 0.05 compared to R6/2 controls. (n=5 and bars represent S.E.M.). Figures 1(B) and 1(C) illustrate a relative expression of FL-, NT- PGC-1α, PPARα, γ, δ, NRF-1, Cyt c, Tfam and ATP synthase in muscle (B) or brown adipose tissue (BAT) (C) of R6/2 mice on a standard diet or on the bezafibrate diet, β-actin and wild-type mice on a standard diet were used as reference.
Figure 2. Bezafibrate improves the behavioral phenotype and extends survival in R6/2 mice. Figure 2(A) illustrates a Grip strength analysis of R6/2 mice and their wild-type littermates on bezafibrate diet. There is a rapidly progressive reduction of muscle strength that is improved in bezafibrate treated R6/2 mice as compared to the R6/2 mice on a standard diet. \( *p < 0.001 \), as compared to the R6/2 controls. (n=8 for each genotype, bezafibrate-or standard diet and bars represent S.E.M.). Figure 2(B) illustrates a measurement of exploratory activity in R6/2 mice at different ages. R6/2 mice are significantly hypoactive as compared to their wild-type littermates \( (*p < 0.001, n=8) \). Bezafibrate significantly restores normal activity and exploration. Figure 2(C) illustrates an assessment of motor coordination in R6/2 mice on bezafibrate diet. R6/2 mice showed progressive, robust deficits on rotarod, with a significantly reduced latency to fall starting at 6 weeks. Bezafibrate treated mice remained on the rotarod longer than the untreated R6/2 mice. Figure 2(D) illustrates a Kaplan-Meier survival plot of R6/2 mice on the bezafibrate diet in comparison to R6/2 mice on a standard diet. No mice in wild-type groups (bezafibrate or standard diet) died in the observed time frame. (n=10 in each group).

Figure 3. Bezafibrate prevents neurodegeneration and increases mitochondrial density. Figure 3(A) illustrates a Calbindin staining in the striatum of 12-week-old R6/2 mice and their wild-type littermates on bezafibrate or standard diet. Figure 3(B) illustrates electron micrographs showing degenerated neurons in the striatum of R6/2 mice (a,b) and its amelioration by Bezafibrate (c,d). a,b: Apoptotic neurons with condensed cytoplasm and abnormal nuclear shape showing margination and condensation of chromatin. The presence of large cytoplasmic vacuoles (bold arrow) and lysosome like dense bodies is also noted. Degenerated mitochondria (light arrow) and lot of empty spaces can also be seen. c,d: In striata from bezafibrate treated R6/2 mice, the cytoplasm of the neuron is preserved and the axonal and dendritic profiles in the neuropil are relatively intact. Scale bars 2μm. Magnification 10,000x. Figure 3(C) illustrates a measurement of mitochondrial density in striatum region of the brain from R6/2 mice and their wild-type littermates on the bezafibrate diet or a standard diet. 10-15 neurons were counted per animal. n=3, \( *p<0.05 \) as compared to wild-type controls. \( §p<0.05 \) compared to R6/2 controls.
Figure 4. Bezafibrate attenuates astrogliosis in R6/2 brains. Photomicrographs show glial fibrillary acidic protein (GFAP) immunoreactivity in the striatum of wild-type and R6/2 mice with or without bezafibrate treatment. GFAP-labeled hypertrophied astrocytes (inset) are evident in the striatum of R6/2 mice. Astroglosis in the HD striatum is reduced by bezafibrate treatment.

Figure 5. Amelioration of oxidative stress in striatum of R6/2 mice by bezafibrate. Figure 5(A) illustrates Malondialdehyde immunostaining in striatum of R6/2 mice and wild-type littermates on bezafibrate or standard diet. Insets show regions at a higher magnification. Figure 5(B) illustrates a Bar-graph showing measurement of MDA levels by high-performance liquid chromatography (HPLC). *p<0.001 as compared to wild-type controls. $p<0.001$ compared to R6/2 controls.

Figure 6. Fiber-type switching and abnormal ultrastructural abnormalities of muscle are reversed by bezafibrate. Figure 6(A) illustrates histochemical staining for succinate dehydrogenase (SDH) in soleus muscle sections from wild-type and R6/2 mice with or without bezafibrate treatment. Figure 6(B) shows quantitation of SDH histochemistry of soleus muscle. Decreased proportion of mitochondria enriched oxidative type I fibers can be seen in soleus from R6/2 mice as compared to wild-type. An enrichment of type I fibers and a decrease in glycolytic type IIB fibers can be seen in soleus muscle from R6/2 mice on bezafibrate diet. *p<0.05 as compared to wild-type controls. $p<0.001$ compared to R6/2 controls. Figure 6(C) illustrates a transmission electron microscopic analysis of soleus muscle from wild-type and R6/2 mice on standard or bezafibrate diet. Panel 'a' shows a micrograph from the soleus muscle of a wild-type mouse. Note the arrangement of mitochondria (M) along the Z-line (white arrow-head). The micrographs shown in panel a, b and c are at taken lower magnification (19,000x), and those in panels d, e, f and g are taken on higher magnification (48,000x). An altered morphology, number and alignment of mitochondria (black arrow) along the Z-lines can be seen in R6/2 mice under basal conditions. Structures resembling an autophagosome could also be noted (black arrow-head). Mitochondria are well organized and appear to be of normal shape and number in soleus muscle of R6/2 mice treated with bezafibrate (c, g).

Figure 7. Bezafibrate reduces BAT vacuolization in R6/2 mice. Brown adipose tissues of wild-type and R6/2 mice stained with hematoxylin-and-eosin showing increased vacuolization in the R6/2 mice. Oil red O staining (red staining, inset) revealed...
abundant accumulation of larger lipid droplets in the R6/2 mice as compared to wild-type mice. Bezafibrate reduces the accumulation of lipids and vacuolization in the R6/2 mice.

Figure 8 shows the arrangement of myofibrils in muscle of a non-disease mouse.

Figure 9 reports the effect of bezafibrate treatment in liver of R6/2 mice and their wild-type littermates. H&E staining of the liver of wild-type and R6/2 mice with or without bezafibrate treatment. Vacuolization of hepatocytes is present in the liver of R6/2 mice at baseline. Liver morphology appears normal in R6/2 mice treated with bezafibrate.

Figure 10. Bezafibrate reduced tau pathology and tau hyperphosphorylation in P301S mice. Fig. 10(A). Immunohistochemical staining with the MCI antibody in the hippocampus and cerebral cortex (scale bar: 100 µm). Fig. 10(B). Number of MCI positive neurons in the hippocampus and cerebral cortex of P301S mice fed a control diet (Tg Control, n=4) and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=5). Fig. 10(C). Immunohistochemical staining with the AT8 antibody to phosphorylated tau in the hippocampus and cerebral cortex (scale bar: 100 µm). Fig. 10(D). Percent area covered by AT8 immunoreactivity in the hippocampus and cerebral cortex of P301S mice fed a control diet (Tg Control, n=4) and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=5). Administration of bezafibrate in P301S mice significantly reduced tau pathology and phosphorylation (Unpaired t-tests, * p<0.05).

Figure 11. Bezafibrate treatment affected GSK3β phosphorylation in P301S mice. Fig. 11(A). Western blots of phospho-GSK3p, total GSK3β, and Fig. 11(B,C) quantification by optical densities in wild-type mice fed a control diet (Wt Cont, n=5), wild-type fed a bezafibrate (Wt Beza, n=2), P301S mice fed a control diet (Tg Cont, n=5) and P301S mice fed a bezafibrate diet (Tg Beza, n=5). Fig. 11(C). Levels of total GSK3β mRNA in wild-type mice fed a control diet (Wt Cont, n=7), wild-type fed a bezafibrate (Wt Beza, n=6), P301S mice fed a control diet (Tg Cont, n=7) and P301S mice fed a bezafibrate diet (Tg Beza, n=7). Bezafibrate treatment affected phosphorylation of GSK3β levels in P301S mice, without affecting total GSK3β mRNA and protein levels (Fisher PLSD, * p<0.05).
Figure 12. Bezafibrate treatment reduced inflammation in P301S mice. Fig. 12(A). Immunohistochemical staining with the CD11b antibody in the hippocampus and cerebral cortex. Fig. 12(B). Intensity (density) of CD11b in P301S mice fed a control diet (Tg Control, n=4) and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=5) (scale bar: 100 μm). Administration of bezafibrate significantly reduced microglial activation in the brains of P301S mice (Unpaired t-tests, * p<0.05). Levels of iNOS (C) and COX2 (F) mRNA in wild-type mice fed a control diet (Wt Control, n=7), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=6), P301S mice fed a control diet (Tg Control, n=7), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=5). Western blots of iNOS (D) and COX2 (G) and their quantifications by optical densities normalized to β-actin (E, H) in wild-type mice fed a control diet (Wt Control, n=4), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=2), P301S mice fed a control diet (Tg Control, n=5), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=5). P301S mice had significantly elevated inflammation relative to their wild-type littermates (Fisher PLSD, † p<0.05). After bezafibrate treatment, both iNOS and COX2 mRNA and protein expression were significantly decreased in P301S mice (Fisher PLSD, * p<0.05).

Figure 13. Bezafibrate treatment improved behavioral deficits in P301S mice. Distance moved (A), rearings (B), and anxiety (time spent in the center) (C) of the open field in wild-type mice fed a control diet (Wt Control, n=16), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=16), P301S mice fed a control diet (Tg Control, n=14), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=13). P301S mice were significantly hyperactive and disinhibited in the open field test as compared to wild-type littermates (Fisher PLSD, † p<0.05). Bezafibrate treatment significantly reduced behavioral abnormalities in P301S mice (Fisher PLSD, * p<0.05).

Figure 14. Bezafibrate treatment increased fatty acid metabolism in P301S brains. Expression of PPAR (A) and fatty β-oxidation genes (B) in the brains of wild-type mice fed a control diet (Wt Control, n=6), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=6), P301S mice fed a control diet (Tg Control, n=6), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=6). P301S mice had reduced mRNA levels of HMGCS2 (Fisher PLSD, † p<0.05). After bezafibrate treatment, there was a trend towards an increase in PPARα and PPARβ, and there was a significant increase of HMGCS2 mRNA levels in P301S mouse brains (Fisher PLSD, * p<0.05).
Bezafibrate treatment prevented lipid vacuoles and activated mitochondrial biogenesis and β-oxidation genes in brown adipose tissue of P301S mice. Body weight of male (A) and female (B) wild-type mice fed a control diet (Wt Control, total n=16), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, total n=16), P301S mice fed a control diet (Tg Control, total n=16), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, total n=15). Bezafibrate treated mice had significantly lower body weight than control treated mice (Fisher PLSD, * p<0.05). (C) Hematoxylin-eosin staining of brown adipose tissue in wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=5), P301S mice fed a control diet (Tg Control, n=5), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=5). (C) Oil red O staining in bottom right panels to verify the presence of lipids. P301S mice had increased size and number of lipid vacuoles, pathology which was improved after bezafibrate treatment. Expression of PPARs (D) and energy metabolism related genes (E, F) in wild-type mice fed a control diet (Wt Control, n=3), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=3), P301S mice fed a control diet (Tg Control, n=6), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=6). There were significant increases in PPARα, PGClα, NRF1, and Tfam in the brown adipose of P301S mice after bezafibrate treatment (Fisher PLSD, * p<0.05). Also, there was a trend toward an increase of PPARγ and Sirt1. (G) Expression of fatty acid β-oxidation genes in wild-type mice fed a control diet (Wt Control, n=3), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=3), P301S mice fed a control diet (Tg Control, n=6), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=6). There was a significant increase in HMGCS2, CPT1A, and ACOX1 in the brown adipose of P301S mice after bezafibrate treatment (Fisher PLSD, * p<0.05).

Bezafibrate treatment affected oxidative stress markers and energy metabolism in P301S mice. Fig. 16(A) Malondialdehyde, Fig. 16(B) protein carbonyls, and 16(C) ratios of GSSG/GSH in wild-type mice fed a control diet (Wt Control, n=6), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=6), P301S mice fed a control diet (Tg Control, n=6), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=6). Fig. 16(D) P301S mice had elevated carbonyls as compared to their wild-type littermates (Fisher PLSD, † p<0.05). Bezafibrate treatment significantly decreased carbonyl and GSSG/GSH levels in the brains of P301S mice (Fisher PLSD, * p<0.05). Fig. 16(D) MtDNA copy number in brains of wild-type mice fed a control diet
(Wt Control, n=6), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=6), P301S mice fed a control diet (Tg Control, n=6), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=6). MitDNA copy number was significantly increased in brains of P301S mice after bezafibrate treatment (Fisher PLSD, * p<0.05). Fig. 16(E) Gene expression of Sirt1, PGCla, NRF1 and Tfam in the brains of wild-type mice fed a control diet (Wt Control, n=6), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=6), P301S mice fed a control diet (Tg Control, n=6), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=6). There was a significant increase of Sirt1 and Tfam mRNA levels in P301S mouse brains after bezafibrate treatment (Fisher PLSD, * p<0.05).

Figure 17. Bezafrate treatment did not affect mitochondrial enzymes in P301S brains. Mitochondrial enzyme activity (A, B) and ATPase protein level (C) in wild-type mice fed a control diet (Wt Control, n=8), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=8), P301S mice fed a control diet (Tg Control, n=8), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=8). Bezafrate did not significantly affect mitochondrial enzyme activity in P301S mice (Fisher PLSD, p>0.05).

Figure 18. Effects of transgene expression of human tau with P301S mutation and bezafibrate treatment on tissue lipid metabolism. Lipid extracts from either brain tissues (A-C) or brown adipose tissue (D-F) were analyzed by LC-MS for different lipid classes. The total lipid composition of individual lipid subclasses was expressed as relative to the wild-type (Wt) control mice for comparative purpose (A, D). The obtained data was further analyzed for phospholipid and diacylglycerol fatty acid chain length (B, E) and degrees of unsaturation (C, F) (Unpaired t-tests, * p<0.05).

Figure 19. Heat map showing statistically significant lipid changes seen in brain tissue of mice with or without the P301S mutant human tau, that were fed control or bezafibrate diets. The degree of change is indicated by the color bar above each heat map and is expressed as a log 2 ratio. In each heat map, the first column shows ratios of wild-type (Wt) bezafibrate relative to Wt controls, second column shows ratios of P301S (Tg) controls relative to Wt controls, third column shows ratios of Wt controls relative to Tg bezafibrate and fourth column shows ratios of Tg controls relative to Tg bezafibrate.

Figure 20. Heat map showing statistically significant lipid changes seen in brown adipose tissue of mice with or without the P301S mutant human tau, injected
intraperitoneally with vehicle or bezafibrate for 4 hours. The degree of change is indicated by the color bar above each heat map and is expressed as a log 2 ratio. In each heat map, the first column shows ratios of wild-type (Wt) treated with bezafibrate relative to Wt controls, the second column shows ratios of P301S (Tg) controls relative to Wt controls, the third column shows ratios of Wt controls relative to Tg bezafibrate, and the fourth column shows ratios of Tg controls relative to Tg bezafibrate.

Figure 21. The human tau protein was not present in brown adipose tissue of P301S mice. Fig. 21(A) Immunohistochemical staining with the HT7 antibody in the brown adipose tissue of wild-type and P301S mice with or without primary antibody (scale bar: 100 μm). Fig. 21(B) Western blots of human tau using the HT7 in the brown adipose tissue of wild-type mice (BAT Wt Control, n=3) and P301S mice (BAT Tg Control, n=3). Brain homogenates of Wt Control (n=1) and Tg Control (n=1) were used as positive controls. Fig. 21(C) Ponceau S staining of the membrane was done as a loading control. No human tau protein was detected in brown adipose tissue of P301S mice.

DETAILED DESCRIPTION OF THE INVENTION

In the following description, reference is made to the accompanying drawings that form a part hereof, and in which is shown by way of illustration specific embodiments which may be practiced. These embodiments are described in detail to enable those skilled in the art to practice the invention, and it is to be understood that other embodiments may be utilized and that logical changes may be made without departing from the scope of the present invention. The following description of example embodiments is, therefore, not to be taken in a limited sense, and the scope of the present invention is defined by the appended claims.

The Abstract is provided to comply with 37 C.F.R. §1.72(b) to allow the reader to quickly ascertain the nature and gist of the technical disclosure. The Abstract is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims.

The present invention provides a method for prevention and treatment of a neurodegenerative disease using a pan-PPAR agonist. In certain embodiments, the pan-PPAR agonist is bezafibrate or an active derivative thereof. In light of the data obtained from the well known and conventional acceptable mouse model of Huntington's disease,
such as R6/2 mice, the present invention provides that bezafibrate improves behavioral phenotype, increased survival, and the induction of the PGC-la signaling pathway, as well as reduces neuropathological features and significant increases mitochondrial density in striatum of R6/2 mice treated with bezafibrate, demonstrating that bezafibrate is an effective neuroprotective agent for treatment of HD.

[0049] Tauopathies are a class of neurodegenerative diseases or effects of CNS trauma characterized by a pathological aggregation of tau protein in the human brain. The best known of these illnesses is Alzheimer’s disease (AD). The present invention provides that the protective effects of the pan-PPAR agonist bezafibrate in P301S mice, the well known and conventional acceptable tauopathy mouse model in the art, were present at various levels. In the brain, bezafibrate induced tau degradation and prevented tau phosphorylation. By downregulating microglia and COX2, bezafibrate also reduced inflammation. In the spinal cord and the brown adipose tissue, bezafibrate acted by inducing the PGCla pathway and mitochondrial biogenesis, which reduced oxidative stress and brown fat vacuolation. Data from initial clinical trials using specific PPAR agonists in AD are also supportive. The present invention for the first provides in vivo evidence that PPAR activation exerts beneficial effects and ameliorates the behavioral and neuropathologic effects of tauopathy. Thus, the use of potent pan-PPAR agonists can lead to improve efficacy in the treatment of neurodegenerative diseases.

[0050] The present invention thus demonstrates that a pan-PPAR agonist, such as bezafibrate, provides neuroprotection in transgenic mouse models of both tauopathy and Huntington’s disease. These transgenic mice have genetic mutations which cause human illness. Bezafibrate exerts neuroprotective effects in these models. These beneficial effects are improved over those previously reported using selective PPAR agonists, such as thiazolidinediones.

[0051] As used herein, the term "pan-PPAR agonist" refers to any molecule that is capable to activate PPAR receptor and/or one or more PPAR receptor subtypes, now known or later discovered, and/or the PPAR-RXR heterodimer, thus, increasing a biological activity regulated through the PPAR receptor and its downstream signal transduction pathways. Molecules that can act as pan-PPAR agonist include endogeneous and/or synethetic PPAR ligands, including, but not limited to, Abs or Ab fragments, fragments or variants of endogenous PPAR receptors, peptides, antisense oligonucleotides,
small organic molecules, etc. The present invention encompasses any PPAR agonists, now known or later discovered, including, but not limited to, PPARα agonists: bezafibrate, and its derivatives, and other fibrates, such as CP-751461, CP868388, GW7647 and WY-14643; PPARγ agonists: the thiazolidinediones (aka glitazones), such as, rosiglitazone, pioglitazone, troglitazone, MCC-555, rivoglitazone, ciglitazone, GW1929, azelacyl PAF, BUT.13, and NSAIDS, such as ibuprofen, fenoprofen, indomethacin, and naproxen; PPARα/γ agonists: glitazars, such as aleglitazar, muraglitazar and tesaglitazar; and PPARβ/δ agonists: GW0742, W501516, and L165.041.

[0052] The identified pan-PPAR agonists treat, inhibit, control and/or prevent, or at least partially arrest or partially prevent, neurodegenerative disorders and/or diseases, such as Huntington's Disease (HD) and tauopathies, such as Alzheimer's disease (AD), and can be administered to a subject at therapeutically effective doses for the inhibition, prevention, prophylaxis or therapy. The pan-PPAR agonists of the present invention comprise a therapeutically effective dosage of a pan-PPAR agonist, a term which includes therapeutically, inhibitory, preventive and prophylactically effective doses of the pan-PPAR agonist of the present invention and is more particularly defined above. Without being bound to any particular theory, applicants surmise that these pharmaceutical pan-PPAR agonists are effective in treatment when administered to a subject suffering from a neurodegenerative disease or disorder. The subject is preferably an animal, including, but not limited to, mammals, reptiles and avians, more preferably horses, cows, dogs, cats, sheep, pigs, and chickens, and most preferably humans.

[0053] The data obtained from the animal model studies can be used in formulating a range of dosages for use in humans and other mammals. The dosage of such a pan-PPAR agonist lies preferably within a range of circulating plasma or other bodily fluid concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any pan-PPAR agonist of the invention, the therapeutically effective dose can be estimated initially from animal studies. A dosage may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (the concentration of the test pan-PPAR agonist that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately
determine useful dosages in humans and other mammals. Levels of a pan-PPAR agonist in plasma may be measured, for example, by high performance liquid chromatography.

[0054] The amount of a pan-PPAR agonist that may be combined with a pharmaceutically acceptable carrier to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. It will be appreciated by those skilled in the art that the unit content of a pan-PPAR agonist contained in an individual dose of each dosage form need not in itself constitute a therapeutically effective amount, as the necessary therapeutically effective amount could be reached by administration of a number of individual doses. The selection of dosage depends upon the dosage form utilized, the condition being treated, and the particular purpose to be achieved according to the determination of those skilled in the art.

[0055] The dosage regime for treating a neurodegenerative disease or condition with the pan-PPAR agonist of the invention is selected in accordance with a variety of factors, including the type, age, weight, sex, diet and medical condition of the patient, the route of administration, pharmacological considerations such as activity, efficacy, pharmacokinetic and toxicology profiles of the particular compound employed, whether a pan-PPAR agonist delivery system is utilized and whether the pan-PPAR agonist is administered as a pro-drug or part of a drug combination. Thus, the dosage regime actually employed may vary widely from subject to subject.

[0056] The pan-PPAR agonist, such as bezafibrate, of the present invention may be formulated by known methods for administration to a subject using several routes which include, but are not limited to, parenteral, oral, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, intercranial, and ophthalmic routes. The individual pan-PPAR agonist may also be administered in combination with one or more other pan-PPAR agonist of the present invention and/or together with other biologically active or biologically inert agents. Such biologically active or inert agents may be in fluid or mechanical communication with the pan-PPAR agonist(s) or attached to the pan-PPAR agonist(s) by ionic, covalent, Van der Waals, hydrophobic, hydrophillic or other physical forces. It is preferred that administration is localized in a subject, but administration may also be systemic.
The pan-PPAR agonist including bezafibrate of the present invention may be formulated by any conventional manner using one or more pharmaceutically acceptable carriers and/or excipients. Thus, the pan-PPAR agonists and their pharmaceutically acceptable salts and solvates may be specifically formulated for administration, e.g., by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration. The pan-PPAR agonists may take the form of charged, neutral and/or other pharmaceutically acceptable salt forms. Examples of pharmaceutically acceptable carriers include, but are not limited to, those described in REMINGTON'S PHARMACEUTICAL SCIENCES (A. R. Gennaro, Ed.), 20th edition, Williams & Wilkins PA, USA (2000).

The pan-PPAR agonists may also take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, controlled- or sustained-release formulations and the like. Such formulations will contain a therapeutically effective amount of the pan-PPAR agonist, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

The pan-PPAR agonists may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form in ampoules or in multi-dose containers with an optional preservative added. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass, plastic or the like. The formulation may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

For example, a parenteral preparation may be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent (e.g., as a solution in 1,3-butanediol). Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may be used in the parenteral preparation.
[0061] Alternatively, the pan-PPAR agonist may be formulated in powder form for constitution with a suitable vehicle, such as sterile pyrogen-free water, before use. For example, a pan-PPAR agonist, such as bezafibrate, suitable for parenteral administration may comprise a sterile isotonic saline solution containing between 0.1 percent and 90 percent weight per volume of the compound. By way of example, a solution may contain from about 0.1 percent to about 20 percent, more preferably from about 0.55 percent to about 17 percent, more preferably from about 0.8 to about 14 percent, and still more preferably about 10 percent of the pan-PPAR agonist, such as bezafibrate. The solution or powder preparation may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Other methods of parenteral delivery of pan-PPAR agonists will be known to the skilled artisan and are within the scope of the invention.

[0062] For oral administration, the pan-PPAR agonist, such as bezafibrate, may take the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents, fillers, lubricants and disintegrants. The tablets or capsules may optionally be coated by methods well known in the art. If binders and/or fillers are used with the pan-PPAR agonist of the invention, they are typically formulated as about 50 to about 99 weight percent of the pan-PPAR agonist. In one aspect, about 0.5 to about 15 weight percent of disintegrant, and particularly about 1 to about 5 weight percent of disintegrant, may be used in combination with the compound. A lubricant may optionally be added, typically in an amount of less than about 1 weight percent of the compound. Techniques and pharmaceutically acceptable additives for making solid oral dosage forms are described in Marshall, SOLID ORAL DOSAGE FORMS, Modern Pharmaceuticals (Banker and Rhodes, Eds.), 7:359-427 (1979). Other less typical formulations are known in the art.

[0063] Liquid preparations for oral administration may take the form of solutions, syrups or suspensions. Alternatively, the liquid preparations may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and/or
preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, perfuming and sweetening agents as appropriate. Preparations for oral administration may also be formulated to achieve controlled release of the compound. Oral formulations preferably contain 10% to 95% compound. In addition, the compounds of the present invention may be formulated for buccal administration in the form of tablets or lozenges formulated in a conventional manner. Other methods of oral delivery of compounds will be known to the skilled artisan and are within the scope of the invention.

[0064] Controlled-release (or sustained-release) preparations may be formulated to extend the activity of the compound and reduce dosage frequency. Controlled-release preparations can also be used to effect the time of onset of action or other characteristics, such as blood levels of the compound, and consequently affect the occurrence of side effects.

[0065] Controlled-release preparations may be designed to initially release an amount of a pan-PPAR agonist, such as bezafibrate, that produces the desired therapeutic effect, and gradually and continually release other amounts of the pan-PPAR agonist to maintain the level of therapeutic effect over an extended period of time. In order to maintain a near-constant level of a pan-PPAR agonist in the body, the pan-PPAR agonist can be released from the dosage form at a rate that will replace the amount of pan-PPAR agonist being metabolized and/or excreted from the body. The controlled-release of a pan-PPAR agonist, such as bezafibrate, may be stimulated by various inducers, e.g., change in pH, change in temperature, enzymes, water, or other physiological conditions or molecules.

[0066] Controlled-release systems may include, for example, an infusion pump which may be used to administer the pan-PPAR agonist(s) in a manner well known and accepted to those of ordinary skill in the art. Typically, using such a system, the pan-PPAR agonist(s) is administered in combination with a biodegradable, biocompatible polymeric implant that releases the pan-PPAR agonist, such as bezafibrate, over a controlled period of time at a selected site. Examples of polymeric materials include polyanhydrides, polyorthoesters, polyglycolic acid, polylactic acid, polyethylene vinyl acetate, and copolymers and combinations thereof. In addition, a controlled release system
can be placed in proximity of a therapeutic target, thus requiring only a fraction of a systemic dosage.

[0067] The pan-PPAR agonists of the invention may be administered by other controlled-release means or delivery devices that are well known to those of ordinary skill in the art. These include, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or the like, or a combination of any of the above to provide the desired release profile in varying proportions. Other methods of controlled-release delivery of pan-PPAR agonists will be known to the skilled artisan and are within the scope of the invention.

[0068] Sustained-release preparations may also be prepared, such as semi-permeable matrices of solid hydrophobic polymers containing the pan-PPAR agonist, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides, copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as injectable microspheres composed of lactic acid-glycolic acid copolymer, and poly-D-(−)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods and may be preferred.

[0069] The pan-PPAR agonist of the present invention can also be entrapped in microcapsules prepared by coacervation techniques or by interfacial polymerization; for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules) or in macroemulsions. The formulations to be used for in vivo administration are highly preferred to be sterile. This is readily accomplished by filtration through sterile filtration membranes or any of a number of techniques.

[0070] The pan-PPAR agonist, such as bezafibrate or others, may also be formulated as a depot preparation. Such long-acting formulations may be administered by implantation (e.g., subcutaneously or intramuscularly or intercranially) or by injection.
Accordingly, the pan-PPAR agonists may be formulated with suitable polymeric or hydrophobic materials such as an emulsion in an acceptable oil or ion exchange resins, or as sparingly soluble derivatives such as a sparingly soluble salt. Other methods of depot delivery of the pan-PPAR agonists will be known to the skilled artisan and are within the scope of the invention.

Various other delivery systems are known in the art and can be used to administer the pan-PPAR agonists of the invention. Moreover, these and other delivery systems may be combined and/or modified to optimize the administration of the pan-PPAR agonists of the present invention.

An effective amount of a pan-PPAR agonist relates generally to the amount needed to achieve a preventive or therapeutic objective, administration rate, and depletion or metabolic rate of the pan-PPAR agonist from a subject. Common ranges for effective doses may be, as a nonlimiting example, from about 0.1 mg/kg body weight to about 50 mg/kg body weight. Dosing frequencies may range, for example, from twice daily to once a week.

The pan-PPAR agonists can be synthesized chemically and/or produced by any suitable methodology and/or technology known to those skilled in the art. Formulations may also contain more than one active pan-PPAR agonist for a particular treatment, preferably those with activities that do not adversely affect each other. The composition may comprise an agent that enhances function, as a nonlimiting example, other types of neuroprotective agents, a cytotoxic agent, cytokine, chemotherapeutic agent or growth-inhibitory agent.

In various embodiments, the present invention can also involve kits. Such kits can include the pan-PPAR agonists of the present invention and, in certain embodiments, instructions for administration. When supplied as a kit, different components of a pan-PPAR agonist formulation can be packaged in separate containers and admixed immediately before use. Such packaging of the components separately can, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the pan-PPAR agonists. The pack may, for example, comprise metal or plastic foil such as a blister pack. Such packaging of the components separately can also, in certain instances, permit long-term storage without losing activity of the
components. In addition, if more than one route of administration is intended or more than one schedule for administration is intended, the different components can be packaged separately and not mixed prior to use. In various embodiments, the different components can be packaged in one combination for administration together.

[0075] Kits may also include reagents in separate containers such as, for example, sterile water or saline to be added to the active component packaged separately. For example, sealed glass ampules may contain purified pan-PPAR agonist and in a separate ampule, sterile water, sterile saline or sterile each of which has been packaged under a neutral non-reacting gas, such as nitrogen. Ampules may consist of any suitable material, such as glass, organic polymers, such as polycarbonate, polystyrene, ceramic, metal or any other material typically employed to hold reagents. Other examples of suitable containers include bottles that may be fabricated from similar substances as ampules, and envelopes that may consist of foil-lined interiors, such as aluminum or an alloy. Other containers include test tubes, vials, flasks, bottles, syringes, and the like. Containers may have a sterile access port, such as a bottle having a stopper that can be pierced by a hypodermic injection needle. Other containers may have two compartments that are separated by a readily removable membrane that upon removal permits the components to mix. Removable membranes may be glass, plastic, rubber, and the like.

[0076] In certain embodiments, kits can be supplied with instructional materials. Instructions may be printed on paper or other substrate, and/or may be supplied as an electronic-readable medium, such as a floppy disc, mini-CD-ROM, CD-ROM, DVD-ROM, Zip disc, videotape, audio tape, and the like. Detailed instructions may not be physically associated with the kit; instead, a user may be directed to an Internet web site specified by the manufacturer or distributor of the kit, or supplied as electronic mail.

[0077] The present invention provides a method for preventing and treating a neurodegenerative disease or disorder using one or more pan-PPAR agonist. In certain embodiments, the neurodegenerative disease is Huntington's disease and/or tauopathies, including, but not limited to, Alzheimer's disease (AD), Pick's complex or Pick' disease or dementia, such as Progressive supranuclear palsy; Dementia pugilistica (chronic traumatic encephalopathy); traumatic encephalopathy; Frontotemporal dementia and parkinsonism linked to chromosome 17; Lytico-Bodig disease (Parkinson-dementia complex of Guam); Tangle-predominant dementia; Ganglioglioma; gangliocytoma;
Meningioangiomatosis; Subacute sclerosing panencephalitis; lead encephalopathy; tuberous sclerosis; Hallervorden-Spatz disease; lipofuscinosis; corticobasal degeneration; Argyrophilic grain disease (AGD); corticobasal degeneration; Frontotemporal dementia; or Frontotemporal lobar degeneration.

EXAMPLES

[0078] The present description is further illustrated by the following examples, which should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, or published patent applications as cited throughout this application) are hereby expressly incorporated by reference.

ABBREVIATIONS

[0079] Peroxisome proliferator activated receptor (PPAR); Peroxisome proliferator activated receptor-y-coactivator 1a (PGC-la); Mitochondrial transcription factor a (Tfam); Cytochrome c (Cyt c); Nuclear respiratory factor-1 (NRF-1); Glutathione peroxidase-1 (Gpx-1); hemoxygenase-1 (HO-1), Nuclear factor (erythroid-derived 2)-like 2 (Nrf-2);

Malondialdehyde (MDA); Brownadipose tissue (BAT).

EXAMPLE 1

BEZAFIBRATE’S EFFECTS ON THE HUNTINGTON DISEASE (HD)

MATERIALS AND METHODS FOR HD WORK

Reagents

[0080] Bezafibrate, malondialdehyde standard (MDA, 98% purity) and other chemicals were purchased from Sigma (St. Louis, MO, USA). R6/2 mice were from Jackson Laboratory, Bar Harbor, USA. Anti-calbindin was from Chemicon, Temecula, CA, USA; anti-malondialdehyde modified protein was a gift from Dr. Craig Thomas and anti-glia fibrillary acidic protein was from Dako, Denmark. The sequences of all the primers used in this study have been published elsewhere and/or are available on request (Chaturvedi et al. 2009; Chaturvedi et al 2010).

Animal treatment

[0081] All experiments were conducted within National Institutes of Health guidelines for animal research and were approved by the Weill Cornell Medical College Animal Care and Use Committee. The animals were kept on a 12-hr light/dark cycle, with food and water available ad libitum. Mice were fed standard diet containing 0.5%
bezafibrate or standard diet (Purina-Mills Richmond, IN, USA), starting right after weaning up to 3 months of age.

Real-Time PCR

Total RNA was isolated from liquid nitrogen snap frozen tissues using Trizol reagent, according to manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Genomic DNA was removed using RNase free DNase (Ambion) in RNA pellets re-suspended in DEPC-treated water (Ambion). Total RNA purity and integrity was confirmed by ND-1000 NanoDrop (NanoDrop Technologies). Equal amounts of RNA were reverse transcribed using the cDNA Synthesis Kit (Invitrogen, USA). Real-time RT-PCR was performed using the ABI prism 7900 HT sequence detection system (Applied Biosystems, Foster City, CA). Expression of the gene β-actin served as a control to normalize values. Relative expression was calculated using the AACt method.

Behavioral tests

Experimenters were blind to the genotype during all testing, at least until the appearance of a robust behavioral phenotype in the mutants. A behavioral testing battery consisting of: rotarod, grip strength and open field were utilized. On the rotarod (Economex, Columbus Instruments, Columbus, OH, USA), mice were tested over 3 consecutive days, in three 5 min-trials, with an accelerating speed (from 0 to 40 RPM in 5 min) separated by a 30-min inter-trial interval. The latency to fall from the rod was recorded. Exploratory behavior was recorded in the open-field (45 cm x 45 cm; height: 20 cm), for 10 min per day using a video tracking system (Ethovision 3.0, Noldus Technology, Attleborough, MA, USA) and averaged over 3 days. For the grip strength test, mice were held by the tail and placed on a wire-grill apparatus so that they grabbed the handle with both front paws and then gently pulled back until they released it. Each session consisted of 5 trials.

Immunohistochemistry

Mice intended for neuropathologic analysis were deeply anesthetized by intraperitoneal injection of sodium pentobarbital and perfused with 0.9% sodium chloride followed by 4% paraformaldehyde. Post-fixation, staining and processing of brain, muscle, BAT and liver samples were performed as described previously (Chaturvedi et al. 2009; Chaturvedi et al 2010; Stack et al 2010).
Transmission Electron Microscopy

[0085] Transmission electron microscopy was performed using previously published methods (13), except that for striatum the post-fixation was performed in 1% Os04- in 0.1M buffer instead of 1% Os04-1.5%-ferricyanide (soleus) for 60 min at room temperature.

High-performance liquid chromatography

[0086] The HPLC determination of MDA was carried out by a method modified from a previous report (Agarwal and Chase 2002). The HPLC system consisted of a Waters 717plus autosampler, 515 isocratic pump and 470 scanning fluorescence detector (Waters, Milford, MA). Pump flow-rate was 1.0 ml/min with mobile phase comprised of acetonitrile-buffer (40:60, v/v). The buffer was 50 mM potassium monobasic phosphate (anhydrous) with an adjusted pH of 6.8 using 5 M potassium hydroxide. The fluorescence detector was set at an excitation wavelength of 515 nm and emission wavelength of 553 nm. The column was an ESA 150 x 3mm C18 column with particle size 3μm (ESA, Inc Chelmsford, MA) placed in a column warmer set to 30℃. Peak heights were integrated by an ESA 501 chromatography data system (ESA, Inc Chelmsford, MA).

RESULTS OF HD WORK

Bezafibrate induces a battery of genes in the PGC-la signaling pathway

[0087] R6/2 mice with an N-terminal genomic fragment containing exon 1 with approximately 130 CAG repeats (Mangiarini et al. 1996) were utilized. R6/2 mice and their wild-type littermates were raised on a diet containing 0.5% bezafibrate or standard chow, starting right after weaning. Quantitative real-time PCR analysis revealed that both the full-length (FL) and N-truncated (NT)-isoforms of PGC-la are downregulated in R6/2 mice brain, muscle and brown adipose tissue (BAT) compared to their wild-type littermates (Fig. 1A, B, C). Both FL and NT-PGC-la isoforms were significantly induced in the brain and peripheral tissues of R6/2 mice that were fed the bezafibrate diet, and their levels were not significantly different from those of the wild-type controls. The ability of PGC-1α to activate a diverse set of metabolic programs in different tissues depends on its ability to form heteromeric complexes with a variety of transcription factors, including nuclear respiratory factor-1 (NRF-1) and PPARs. PPAR-γ and PPAR-δ, are co-activated with PGC-1α in a positive feedback loop, and regulate glucose metabolism, fatty acid
oxidation and mitochondrial biogenesis (Hondares et al. 2006; Huss and Kelly 2004). The mRNA expression levels of the three isoforms of PPARs (α, γ and δ) and that of Cytochrome c (Cyt c), Tfam, ATP synthase were lower in the R6/2 brain, muscle and BAT as compared to their wild-type littermates, however, levels of NRF-1 were unchanged in brain and BAT. Bezafibrate administration restored the mRNA levels of PGC-1α, PPARs and downstream targets of PGC-1α, Cyt c, Tfam and ATP synthase in brain, muscle and BAT of R6/2 mice (Fig. 1).

Concomitant with the stimulation of expression of genes involved in mitochondrial energy production, PGC-1α also induces genes responsible for countering reactive oxygen species (ROS) generated as by-products of oxidative metabolism (St-Pierre et al. 2006; Lin et al. 2005). In R6/2 brains, it was found that genes responsive to ROS, such as, hemoxygenase-1 (HO-1), Nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) and Gpx-1 were significantly downregulated, and bezafibrate restored the levels of these genes to control levels in the R6/2 mice (Fig. 1A).

**Improved behavior and survival in R6/2 mice treated with bezafibrate**

To assess muscle strength the R6/2 mice and their wild-type littermates were tested on the grip strength test (Fig. 2A). As reported previously, R6/2 mice showed a robust deterioration of their grip strength as they aged, compared to their wild-type littermates with differences being significant at 8, 10 and 12 weeks of age (Menalled et al. 2009). Bezafibrate treated R6/2 mice showed an increase in grip strength (up to 2 fold) compared to the untreated R6/2 mice (Fig. 2A). To further assess motor function, general activity and exploration, mice were tested in the open field test. R6/2 transgenic mice were significantly hypoactive, as measured by the total distance covered, and in the bezafibrate treated R6/2 group, a significant amelioration of the deficit was seen. The total distance covered was significantly greater in the treated group than in the R6/2 mice on a standard diet (Fig. 2B). Motor coordination was assessed by performance on an accelerated rotarod apparatus. Latency to fall was recorded for 3 trials per weekly assessment and scores were averaged (Fig. 2C). Consistent with previous literature, R6/2 mice showed progressive, robust deficits on rotarod, with a significantly reduced latency to fall starting at 6 weeks of age (Menalled et al. 2009). Bezafibrate treated mice remained on the rotarod longer than the untreated R6/2 mice, indicating better motor coordination in the treated mice (Fig. 2C).
R6/2 mice normally die prematurely as compared to their wild-type
littermates, between the age of 70-91 days (Mangiarini et al. 1996). Symptomatic mice
approaching the disease endstage were examined twice daily (morning and late afternoon)
to assess when they reached endstage of the disease, as assessed by the righting reflex or
failure to eat moistened chow placed beside mice over a 24 hour period. At this point,
mice were euthanized by CO2 inhalation. This day was recorded as time of death of the
mouse. Figure 2D shows a Kaplan-Meier plot of the survival of R6/2 mice. In our hands, the
R6/2 mice survived to 77-88 days of age, and the longest living mouse died at 90 days of
age (mean=84 ± 1.2 days). Bezafibrate treated R6/2 mice lived 20% longer than the R6/2
mice on a standard diet (mean=102 ± 3.2 days) (Fig. 2D).

Bezafibrate rescues neuropathological features in R6/2 mice

The neuropathological features of HD are general atrophy of the brain, with
losses of projection neurons in the deeper layers of the cortex and calbindin
immunoreactive medium spiny neurons (MSNs) in the caudate-putamen (Vonsattel and
DiFiglia 1998). A stereological analysis of calbindin-immunoreactive medium spiny
neuronal perikarya was performed in the striatum of 12-week-old R6/2 mice. Consistent
with other studies, a reduction of neuron size was found in R6/2 mice as compared to
wild-type controls (Fig. 3A), (Ferrante et al 2002). Induction of PGC-1α expression by
bezafibrate treatment was accompanied by increases in the calbindin-positive neuron area
in the R6/2 mice.

The striatum of R6/2 mice treated with bezafibrate and those on standard
diet were further examined at the ultrastructural level. Several apoptotic neurons with
condensed cytoplasm and abnormal nuclear shape showing margination and condensation
of chromatin were observed (Fig. 3B-A,B). Enlarged extracellular spaces, cytoplasmic
vacuoles and lysosome-like dense bodies were also noted. Moreover, degenerated or
degenerating mitochondria could also be seen. Ultrastructural abnormalities in the brains
of HD mice have previously been noted, including the presence of dark neurons and
abnormally shaped nuclei (Davies et al 1997; Yu et al 2003). In the bezafibrate treated
R6/2 mice, amelioration of these abnormalities was noted. In particular, in the striata from
bezafibrate treated R6/2 mice, the cytoplasm of the neurons is preserved, and the axonal
and dendritic profiles in the neuropil are relatively intact (Fig. 3B-c,d). Mitochondrial
density was also measured. For this purpose, the intact mitochondria count per cell was
noted and divided by the area of the cytoplasm, yielding the mitochondrial density (Fig. 3C). Several neurons were counted per animal and a group average is presented in Fig. 3C. A significant increase in numbers of mitochondria in the striata of bezafibrate treated R6/2 mice was seen, as compared to the R6/2 mice on standard chow.

Evidence for amelioration of oxidative stress in R6/2 mice treated with bezafibrate

In HD, the generation of ROS and the resulting oxidative stress, are implicated in the neurodegeneration and neuronal death (Browne et al 1997; Reviewed in Stack et al 2008). Postmortem human HD brain tissue shows increased levels of oxidative damage markers, including increased cytoplasmic lipofuscin, DNA strand breaks, oxidized DNA bases, protein nitration, carbonyls and lipid oxidative damage. Levels of malondialdehyde (a marker for oxidative damage to lipids), are elevated in human HD striatum and cortex as compared with age-matched controls (Browne et al 1997). Increased immunoreactivity for MDA was observed in R6/2 striatum, which was ameliorated by the bezafibrate diet (Fig. 5A). Consonant with the immunohistochemical data, HPLC analysis also revealed elevated levels of MDA in R6/2 brains which were significantly reduced with bezafibrate treatment (Fig. 5B).

Bezafibrate prevents the fiber-type switching and structural abnormalities in muscle

Muscle fibers can be classified as 'slow-twitch' fatigue resistant fibers which are dependent on PGC-la, and contain numerous mitochondria and use oxidative phosphorylation to generate ATP (type I and IIA fibers), and 'fast-twitch fatigueable' fibers (type IIx and IIB fibers), which have few mitochondria and which utilize glycolysis to generate ATP (Lin et al. 2002). PGC-la levels are normally high in muscle enriched with type I fibers, such as the soleus muscle, and very low in type II fiber rich muscles such as the extensor digitorum longus and the gastrocnemius (Lin et al. 2002). The
soleus muscle of R6/2 mice and their wild-type littermates for fiber typing were examined using succinate dehydrogenase (SDH) histochemistry (Fig. 6A). There was reduced SDH staining in the soleus of R6/2 mice on a standard diet. Quantitation of the SDH histochemistry revealed a significant reduction of type I fibers, and an increase in type II fibers in the soleus muscle of R6/2 mice, consistent with the reduced expression of PGC-1α. A reversal of this fiber-type switching was seen in R6/2 mice on the bezafibrate diet, with the type I and II fibers returning back to normal levels seen in wild-type mice (Fig. 6B).

Further studies to determine the effects of bezafibrate on mitochondrial area and mitochondrial number were carried out using electron microscopy. In wild-type mice, mitochondria are uniform in size and align regularly along the Z lines (Fig. 6C), whereas in the R6/2 mice, the mitochondria are irregular in shape and poorly aligned. Similarly, PGC-1α-deficient mice show fewer and smaller mitochondria in soleus muscle (Leone et al. 2005). In the R6/2 mice treated with bezafibrate, mitochondria appeared to be of normal shape and their arrangement along the Z-lines was restored to normal (Fig. 6C).

**Bezafibrate attenuated vacuolization in the brown adipose tissue of R6/2 mice**

Using other mouse models of HD, (N171-82Q and NLS-N171-82Q HD mice), it was previously reported that HD mice have an impaired response to cold temperature, i.e. defective adaptive thermogenesis (Weydt et al. 2006; Chaturvedi et al. 2010). In rodents, brown adipose tissue (BAT) is the principal tissue that mediates the adaptive thermogenesis, and is distinguished from white fat by its high degree of vascularization and mitochondrial density (Cannon and Nedergaard 2004). PGC-1α is expressed in BAT and is a key mediator of adaptive thermogenesis by activating uncoupling protein 1 (Puigserver et al 1998). As seen with the other HD mice models, the H&E staining of BAT from the R6/2 mice showed marked reductions in cell density and nuclei numbers and increased vacuolization when compared with wild-type mice (Fig. 7). The white-fat like appearance of BAT was due to accumulation of neutral lipids as revealed by Oil red O staining (Fig. 7, inset). Bezafibrate reduced vacuolization in the brown adipose tissue of the R6/2 transgenic mice as compared to R6/2 mice fed a standard diet, and reduced oil red O staining was also observed (Fig. 7).

**DISCUSSION OF HD WORK**
A number of bioenergetic and metabolic impairments are known to occur in HD patients: 1) increased lactate production in cerebral cortex and basal ganglia; 2) reduced phosphocreatine to inorganic phosphate ratio in resting muscle, the extent of which correlates with CAG repeat expansion length, and which is exacerbated after exercise; 3) abnormal mitochondrial membrane depolarization in lymphoblasts; 4) impaired complex II, III and IV activity of the mitochondrial oxidative phosphorylation pathway and reducedaconitase activity in the basal ganglia; 5) abnormal ultrastructure of mitochondria in cortical biopsies obtained from patients with both juvenile and adult-onset HD; and 6) pathologic grade dependent reductions in numbers of mitochondria (Kim et al 2010; Reviewed in Browne and Beal 2004). Literature shows that the phenotypic and neuropathologic features of HD can be modeled in rodents and primates, with the mitochondrial toxin 3-nitropropionic acid (Beal et al 1993; Brouillet et al 1995).

Impaired expression and/or function of PGC-1α, the master co-regulator of mitochondrial biogenesis, has been implicated in the pathogenesis of several neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, and HD. A link of the transcriptional coactivator PGC-1α to HD pathogenesis was first suggested by observations in PGC-la-deficient mice (Lin et al. 2004; Leone et al. 2005). PGC-la knockout mice exhibit impaired mitochondrial function, hyperkinetic movement disorder, and striatal degeneration, all features also observed in HD (Lin et al. 2004; Leone et al. 2005). Furthermore, impaired PGC-1a function and levels occur in striatal cell lines, transgenic mouse models of HD and in postmortem brain tissue from HD patients (Cui et al 2006; Weydt et al. 2006). Recent studies showed that expression of mutant htt in primary oligodendrocytes results in decreased expression of PGC-la, and decreased expression of myelin basic protein (MBP) and deficient myelination were found in the R6/2 mouse model of HD (Xiang et al. 2011). A decrease in MBP and deficient postnatal myelination occurs in the striatum of PGC-1a knockout mice (Xiang et al. 2011). In accordance with earlier studies, the PGC-1a signaling pathway is shown to be downregulated in the brain, muscle and brown adipose tissue of R6/2 HD mice. Spongiform lesions in R6/2 striatum, along with the presence of astrogliosis, was also found, which is similar to observations made in PGC-la-deficient mice (Lin et al. 2004; Leone et al. 2005). The neuropathologic observation of spongiform degeneration is
of interest, since similar lesions occur in MnSOD null mice (Hinerfeld 2004), and PGC-la plays an important role in controlling expression of MnSOD (St-Pierre et al. 2006).

[00100] In the present invention, it was found that the impairment of PGC-la pathway can be reversed in brain, muscle and brown adipose tissue from R6/2 HD mice, using the PPAR-pan-agonist, bezafibrate, which was previously shown to be effective in increasing life span and delaying the onset of symptoms in a mouse model of mitochondrial myopathy (Wenz et al 2008). Wide-spread beneficial effects of bezafibrate on behavior, survival and histopathological features in brain, muscle and BAT of R6/2 mice were found. The increase in survival observed with the bezafibrate diet (20%) is comparable to the highest range of percent increases in survival seen in other therapeutic trials in mouse models of HD (Hersch and Ferrante 2004). It was shown that administration of creatine or triterpenoid compounds to N171-82Q mice increased survival by 19% or 21.9% respectively, and that administration of coenzyme Q10 with remacemide or administration of mithramycin increased survival by 31.8% or by 29.1% in R6/2 mice, respectively (Andreassen et al. 2001; Beal and Ferrante 2004; Ferrante et al. 2004; Stack et al 2010). Bezafibrate, therefore, improves the behavioral phenotype and survival of R6/2 mice in a comparable range to the best therapeutic interventions thus far tested. Recently, administration of a PPARy agonist, thiazolidinedione, was shown to produce beneficial effects on weight loss, mhtt aggregates and global ubiquitination profiles in R6/2 mice (Chiang et al. 2010). Earlier, it was shown in STHdhQ111 cells, that PPARy activation by rosiglitazone prevents the mitochondrial dysfunction and oxidative stress that occurred when mutant striatal cells were challenged with pathological increases in calcium (Quintanilla et al 2008).

[00101] It was also found that the improved behavioral phenotype, increased survival and the induction of the PGC-la signaling pathway was accompanied by reduced neuropathological features and a significant increase in mitochondrial density in striatum of R6/2 mice treated with bezafibrate. PGC-la plays a critical role in mitochondrial biogenesis, and in studies of cortical, midbrain and cerebellar granule neurons, both PGC-la and PGC-Iβ control mitochondrial density (Wareski et al 2009). Overexpression of PGC-Iβ or PGC-la, or activation of the latter by SIRT1, protects neurons from mutant htt-induced loss of mitochondria and cell death (Wareski et al 2009). The SIRT1 activator, resveratrol, increases the activity of PGC-la and improves mitochondrial activity as a
consequence of its deacetylation of PGC-1α, which increases its effects on liver, fat and muscle metabolism (Lagouge et al. 2006). Recently, it showed that resveratrol treatment of the N171-82Q transgenic mice, produced increased PGC-1α and reduced vacuolization in BAT and reduced glucose levels, but there were no beneficial effects in the striatum due to poor brain penetration (Ho et al 2010).

[00102] In disease-free neurons, the generation of ROS is a normal by-product of cellular respiration, mediated by mitochondria. Accumulation of ROS in neurons and subsequent oxidative stress is attenuated by free radical scavengers, such as glutathione and superoxide dismutase, preventing subsequent damage (Browne and Beal 2006; Beal 1999). There is evidence for oxidative damage in HD (Reviewed in Stack et al 2008). Markers of oxidative damage, including heme oxygenase (an inducible isoform that occurs in response to oxidative stress), 3-nitrotyrosine (a marker for peroxynitrite-mediated protein nitration), and malondialdehyde (MDA, a marker for oxidative damage to lipids), are elevated in human HD striatum and cortex as compared with age-matched control brain specimens (Browne et al 1999). The extent and intensity of these markers mirror the dorso-ventral pattern of progressive neuronal loss in the neostriatum, with increased immunoreactive expression in the dorsal striatum as compared with the less severely affected ventral striatum. Consistent with the immunohistochemical data, analysis of biochemical assays in HD patients show significant increases in malondialdehyde and 4-hydroxynonenal brain levels, almost 8-fold greater than in control subjects (Stoy et al 2005).

[00103] PGC-1α plays a role in the suppression of oxidative stress, and it induces antioxidant enzymes, including copper/zinc superoxide dismutase (SOD1), manganese SOD (SOD2), and glutathione peroxidase (Gpx1) (St-Pierre et al 2006). In concert with the increase in PGC-1α expression, it was observed that the oxidative stress response genes such as hemeoxygenase-1 (HO-1), Nrf-2 and Gpx1 were also increased in the brains of R6/2 mice treated with bezafibrate. The levels of MDA measured by HPLC, and MDA immunoreactivity in striatum were significantly reduced in R6/2 mice by bezafibrate treatment as compared to the control R6/2 mice. These observations provide strong evidence for amelioration of oxidative stress in R6/2 mice by upregulation of PGC-1α using bezafibrate. Recently, it showed that administration of triterpenoids, which activate
the Nrf2/ARE transcriptional pathways, are neuroprotective in the N171-82Q transgenic mouse model of HD (Stack et al 2010).

PGC-1α is reduced in muscle from HD transgenic mice and in muscle biopsies and myoblasts from HD patients (Chaturvedi et al 2009). There was an impaired response to guanidinopropionic acid (GPA) treatment, in the muscle and brains of NLS-N171-82Q HD mice. In wild-type mice, GPA treatment activated AMPK, which increased PGC-1α, NRF1 and Tfam, and this was accompanied by an increase in COX II/18s rRNA, consistent with mitochondrial biogenesis, increased mtDNA and increased numbers of mitochondria. This pathway, which leads to an increase in mitochondria in response to an energetic stress, was blocked in the NLS-N171-82Q HD mice (Chaturvedi et al. 2009; Chaturvedi et al 2010). Bezafibrate treatment in R6/2 mice rescued the PGC-1α signaling pathway and restored the levels of downstream target genes involved in mitochondrial function, e.g., cytochrome c, Tfam and ATP synthase. Bezafibrate also reversed the fiber type switching back to normal and restored the normal morphology of muscle, shape, numbers and arrangement of mitochondria along the Z-lines in the soleus muscle of R6/2 mice.

PGC-1α is rapidly induced in response to cold exposure and regulates key components of adaptive thermogenesis including the uncoupling of respiration via uncoupling proteins (UCP-1), resulting in heat production in brown adipose tissue (BAT). Significant hypothermia at both baseline and following cold exposure was found in both N171-82Q and R6/2 HD mouse models (Weydt et al. 2006). Following cold exposure, UCP-1 expression is decreased in BAT from N171-82Q transgenic HD mice relative to wild type controls. In brown fat adipocytes, there are reduced ATP/ADP ratios and mitochondrial numbers, similar to the findings in PGC-1α KO mice (Lin et al. 2004; Leone et al. 2005). Similar to previous findings, it was also observed that in BAT of the R6/2 mice, there is a marked vacuolization, which is due to accumulation of neutral lipids. Bezafibrate reduced the vacuolization and oil red O staining in the BAT of R6/2 mice, indicating amelioration of neutral lipid accumulation.

The important role of PGC-1α in the regulation of mitochondrial function, together with the association of mitochondrial dysfunction with HD pathogenesis, implies that activation of PGC-1α may be useful in the treatment of HD. In the present invention, stimulation of PPAR-PGC-1α axis by bezafibrate produces wide-spread beneficial effects
in brain and peripheral tissues of R6/2 model of HD. Bezafibrate is an attractive agent for clinical studies since it has been used in man for more than 25 years, and it is well-tolerated with few side effects. It is therefore particularly attractive for clinical trials in neurodegenerative diseases such as HD. In other work, it was found that bezafibrate exerts beneficial effects in BACHD mice, a full length mhtt transgenic mouse model of HD (Gray et al. 2008; Johri and Beal, unpublished observations). The present invention demonstrating beneficial effects of bezafibrate in the R6/2 mouse model of HD, provides strong evidence that bezafibrate can be proved to be an effective neuroprotective agent for treatment of HD.

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EXAMPLE 2
BEZAFIBRATE ADMINISTRATION IMPROVES BEHAVIORAL DEFICITS AND TAU PATHOLOGY IN P301S MICE

Peroxisome proliferator-activated receptors (PPARs) are ligand mediated transcription factors, which control both lipid and energy metabolism and inflammation pathways. PPARγ agonists are effective in the treatment of metabolic diseases and, more recently, neurodegenerative diseases, in which they show promising neuroprotective effects. The effects of the pan-PPAR agonist bezafibrate on tau pathology, inflammation, lipid metabolism, and behavior were studied in transgenic mice with the P301S human tau mutation, which causes familial frontotemporal lobar degeneration. Bezafibrate treatment significantly decreased tau hyperphosphorylation and the number of MCI-positive neurons. Bezafibrate treatment also diminished microglial activation and expression of both inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2). Additionally, the drug differentially affected the brain and brown fat lipidome of control and P301S mice, preventing lipid vacuoles in brown fat. These effects were associated with behavioral improvement, as evidenced by reduced hyperactivity and disinhibition in the P301S mice. Bezafibrate therefore exerts neuroprotective effects in a mouse model of tauopathy, as shown by decreased tau pathology and behavioral improvement. Since bezafibrate was given to the mice before tau pathology had developed, the data suggest that bezafibrate exerts a preventive effect on both tau pathology and its behavioral consequences. Bezafibrate is therefore a promising agent for the treatment of neurodegenerative diseases associated with tau pathology.

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that act as ligand-dependent transcription factors. PPARα, β, and γ are the three known PPAR isotypes. PPARα is predominantly expressed in the liver, kidney, muscle, adipose tissue, heart, and, to a lesser extent, brain, whereas PPARβ is found in the brain, adipose tissue, and skin and PPARγ is expressed ubiquitously (1). These transcription factors have been linked to lipid transport, metabolism, and inflammation pathways (1). Because of this, synthetic PPAR agonists have been generated as therapeutic agents for the treatment of diabetes and metabolic diseases (2, 3). PPARs have effects on
metabolism and inflammation in both the central nervous system and peripheral tissues, suggesting that they may also play a role in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease (AD) (4).

[00238] Prior reports demonstrated beneficial effects of PPARy agonists, such as thiazolidinediones (TZD) (5), in models of AD (6-9), Parkinson's disease (PD) (10), amyotrophic lateral sclerosis (11, 12) and Huntington's disease (HD) (13, 14). Fibrates, such as fenofibrate (15), are another class of PPAR agonists that primarily target the PPARα pathway, with smaller effects on PPARβ and PPARγ (16-18). Fenofibrate has shown promising neuroprotective effects in models of neurodegenerative diseases including PD (19) and brain injury (20). Interestingly, the neuroprotective effects of PPAR agonists occur through mechanisms involving a reduction of oxidative stress and inflammation (6-9, 20).

[00239] Increased phosphorylation and accumulation of tau within neurons are important pathologic hallmarks of AD and tauopathies. Neurofibrillary tangles are more strongly linked to the cognitive impairment occurring in AD, than is the deposition of β-amyloid (Aβ) (21, 22). Previous reports showed that in vivo PPARγ agonists reduce Aβ and tau phosphorylation in mouse models of AD (23, 24). In CHOtau4R cells, a model of tauopathy, administration of troglitazone also reduced tau phosphorylation (25). In the present study, whether the pan-PPAR agonist bezafibrate exerts beneficial effects in the P301S transgenic mouse model of tauopathy was investigated. Bezafibrate is similar to other fibrates in that it predominantly activates PPARα, but also acts on PPARβ and PPARγ (26). Although PPAR agonists have been linked to activation of PGC1α and mitochondrial biogenesis, activation of AMPK produces fatty acid oxidation by activating both PPARα and PGC1α and PGC1β in cardiac muscle, which maintains mitochondrial substrate oxidation and respiration (27, 28). It was recently shown that bezafibrate had neuroprotective effects in a mouse model of HD (29). The P301S transgenic mice, which express the human tau gene with the P301S mutation, develop progressive tau pathology, accompanied by microglial activation (30, 31), synaptic damage (31), and behavioral impairments (32, 33). In the present study, these mice were treated with 0.5% bezafibrate in the diet from 1 to 10 months of age, and its effects on tau pathology, markers of inflammation, lipid metabolism and behavior are assessed.
RESULTS

Bezafibrate treatment reduced tau pathology and tau hyperphosphorylation in P301S mice

To assess tau pathology in the brains of P301S mice, two mouse monoclonal antibodies were used. MCI is an anti-human tau antibody and an indicator of early tau pathology related to conformational changes prior to the appearance of paired helical filamentous tau (PHF) (N-terminal conformational change, Exon 10, amino-acids 5-15 and 312-322). MCI immunoreactivity appears as non-filamentous cytoplasmic staining in pre-tangle bearing neurons (34, 35). AT8 is another anti-human tau antibody that detects PHF-like tau. The epitope of AT8 (around residue 200) is located outside the region of internal repeats and requires the phosphorylation of serines 199 and/or 202 (pSer202/Thr205) (36). An increase in such markers have been previously described in Alzheimer's disease and used to evaluate tau pathogenesis (37, 38).

As wild-type mice did not show any tau pathology, MCI and AT8 immunostaining were quantified only in the P301S mice. In 10 month-old P301S mice, MCI and AT8 immunoreactivity was markedly increased in the hippocampus and cerebral cortex as compared to their wild-type littermates (Fig. 10). Bezafibrate treatment improved both early tau pathological conformational changes and tau hyperphosphorylation, measured by MCI (Fig. 10A,B) and AT8 (Fig. 10C,D), respectively.

Bezafibrate treatment affected GSK3β phosphorylation in P301S mice

The expression of glycogen synthase kinase-3-beta (GSK3P), a well-characterized serine/threonine protein kinase, which phosphorylates cellular substrates such as the protein tau, and is thought to play a major role in tau phosphorylation in vivo (39-41) were studied. Administration of bezafibrate significantly decreased protein levels of phospho-GSK3P in P301S mice (Fig. 11A,B). Furthermore, it was verified that these effects were not due to an overall inhibition of GSK3β by measuring total GSK3β mRNA and protein levels, which were not altered by bezafibrate treatment in both P301S mice and their wild-type littermates (Fig. 11C,D). It is noted that no significant changes were seen between P301S mice and their wild-type littermates at baseline regarding levels of GSK3β.

Bezafibrate treatment reduced inflammation in P301S mice
Previous reports showed that P301S mice develop early microglial activation as compared to their wild-type littermates (30, 31). In this study, increased microglial activation was observed in 10 month-old P301S mice relative to non-transgenic littermates, as evidenced by elevated CD11b immunoreactivity in the hippocampus and cerebral cortex (Fig. 12A). Bezafibrate treatment significantly decreased CD11b staining intensity associated with activated microglia in P301S mice (Fig. 12B).

PPAR activation is known to inhibit transcriptional regulation of several key inflammatory genes (4). Also, considering the important role of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2) in the pro-inflammatory responses of microgla, their levels were measured after bezafibrate treatment. Both iNOS mRNA and protein levels were significantly reduced in P301S mice fed a bezafibrate diet relative to P301S mice fed a control diet (Fig. 12C-E). Similarly, COX2 mRNA and protein levels were decreased after bezafibrate treatment in the P301S mice (Fig. 12F-H).

Bezafibrate treatment improved behavioral deficits in P301S mice.

The open field test was used to assess locomotion and exploration. In this test, P301S mice were hyperactive relative to their wild-type littermates at 5, 7, and 9 months of age (Fig. 13A,B). P301S mice were also disinhibited as evidenced by the increased time spent in the central area of the apparatus (Fig. 13C). Both locomotor and anxiety-related abnormalities were improved in P301S mice a fed bezafibrate diet as compared to P301S mice fed a control diet (Fig. 13C).

Bezafibrate treatment affected oxidative stress markers and energy metabolism in P301S mice.

It has previously shown that P301S mice have increased protein carbonyl levels starting at 7 months of age (32). Here, oxidative stress were assessed by measuring levels of malondialdehyde, a marker of lipid peroxidation (Fig. 16A), ratios of oxidized (GSSG) to reduced (GSH) glutathione (Fig. 16C), and protein carbonyls in the brains of P301S mice (Fig. 16B). Levels of both malondialdehyde and protein carbonyls were increased in 10 month old P301S mice relative to their wild-type littermates (Fig. 16AB). However, bezafibrate treatment did not affect lipid peroxidation (Fig. 16A). Both protein carbonyls and glutathione levels in P301S mice fed a bezafibrate diet were significantly reduced as compared to P301S mice fed the control diet (Fig. 16BC), although levels of
glutathione were not significantly increased between P301S and wild-type littermates at baseline.

In order to understand the mechanisms in which bezafibrate affected oxidative stress, energy metabolism pathways were examined. First, the mtDNA copy number was analyzed. There was a trend towards a decrease of mtDNA copy number in P301S mice as compared to their wild-type littermates. It was found that this level was elevated after bezafibrate treatment, suggesting that bezafibrate may have induced mitochondrial biogenesis and energy metabolism (Fig. 16D). Furthermore, gene expression of PPAR downstream targets involved in energy metabolism, such as sirtuin 1 (Sirtl), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1a), nuclear respiratory factor 1 (NRF1), and mitochondrial transcription factor A (Tfam) were assessed. No differences were found in these genes at baseline between P301S mice and their wild-type littermates (Fig. 16E). However, after bezafibrate treatment, mRNA levels of Sirtl and Tfam were significantly increased in P301S mice (Fig. 16E).

Further valuation on whether this could be attributed to a mitochondrial antioxidant mediated response was performed. It was shown that bezafibrate treatment did not affect the enzymatic activities of either glutathione reductase or superoxide dismutase (Fig. 17A). Enzymatic activities of other mitochondrial enzymes, such as aconitase, citrate synthase, complex I, succinate dehydrogenase, isocitrate dehydrogenase, and malic enzyme (Fig. 17B), and protein levels of ATPase (Fig. 17C) were also checked. No significant differences in these mitochondrial enzymes were observed with or without bezafibrate treatment in either P301S mice or their wild-type littermates.

Bezafibrate treatment increased fatty acid β-oxidation in P301S brains

In the brains of P301S mice, there was a trend for an increase in both PPARα and PPARβ gene expression (Fig. 14A). In addition, their downstream targets which are involved in fatty acid β-oxidation pathways, such as peroxisomal acyl-coenzyme A oxidase 1 (ACOX1), carnitine-palmitoyl transferase 1A (CPT1A), and 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (HMGCS2) were also examined. After bezafibrate treatment, mRNA levels of HMGCS2 were significantly increased in P301S mice (Fig. 14B).
To determine whether these effects had functional consequences on fatty acid content, types and levels of free fatty acids were studied in the brains of P301S mice (Table 1). At baseline, no significant differences in saturated, mono-unsaturated or total fatty acid levels in P301S mice were detected, as compared to their wild-type littermates. However, P301S mice had elevated levels of poly-unsaturated fatty acids as compared to wild-type mice, as evidenced by a significant increase in linoleic acid (C18:2) (Table 1). After bezafibrate treatment, the level of linoleic acid was significantly reduced in both wild-type and P301S mice (Table 1). There was also a trend towards a decrease of other poly-unsaturated fatty acids in P301S mice fed the bezafibrate diet relative to P301S mice fed a control diet, such as linolenic acid (C18:3) and eicosapentaenoic acid (C20:5).

Table 1

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Wt Control (n=8)</th>
<th>Wt Beza (n=7)</th>
<th>Tg Control (n=8)</th>
<th>Tg Beza (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>1 ± 0.7</td>
<td>1 ± 0.1</td>
<td>1 ± 0.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>14:0</td>
<td>7 ± 0.4</td>
<td>7 ± 0.6</td>
<td>7 ± 0.2</td>
<td>7 ± 0.4</td>
</tr>
<tr>
<td>16:0</td>
<td>245 ± 14</td>
<td>247 ± 17</td>
<td>247 ± 8</td>
<td>249 ± 25</td>
</tr>
<tr>
<td>18:0</td>
<td>408 ± 26</td>
<td>397 ± 24</td>
<td>384 ± 17</td>
<td>399 ± 46</td>
</tr>
<tr>
<td>20:0</td>
<td>3 ± 0.2</td>
<td>3 ± 0.2</td>
<td>3 ± 0.1</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>22:0</td>
<td>2 ± 0.2</td>
<td>2 ± 0.2</td>
<td>2 ± 0.1</td>
<td>2 ± 0.1</td>
</tr>
<tr>
<td>24:0</td>
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<td>2 ± 0.1</td>
<td>2 ± 0.2</td>
<td>2 ± 0.2</td>
</tr>
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<td>26:0</td>
<td>0.2 ± 0.01</td>
<td>0.2 ± 0.01</td>
<td>0.2 ± 0.01</td>
<td>0.2 ± 0.01</td>
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</tr>
<tr>
<td>14:1</td>
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<td>0.2 ± 0.03</td>
<td>0.3 ± 0.04</td>
</tr>
<tr>
<td>16:1</td>
<td>13 ± 1</td>
<td>15 ± 1</td>
<td>12 ± 0.5</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>18:1</td>
<td>191 ± 9</td>
<td>201 ± 15</td>
<td>183 ± 5</td>
<td>190 ± 17</td>
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<td>16 ± 1</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>22:1</td>
<td>5 ± 0.4</td>
<td>5 ± 0.5</td>
<td>5 ± 0.3</td>
<td>5 ± 0.5</td>
</tr>
<tr>
<td>24:1</td>
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<td>4 ± 0.4</td>
<td>4 ± 0.4</td>
<td>4 ± 0.6</td>
</tr>
<tr>
<td>26:1</td>
<td>0.4 ± 0.03</td>
<td>0.4 ± 0.03</td>
<td>0.4 ± 0.05</td>
<td>0.4 ± 0.06</td>
</tr>
<tr>
<td>Poly-unsaturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2</td>
<td>9 ± 0.4</td>
<td>7 ± 0.4</td>
<td>10 ± 0.6</td>
<td>7 ± 0.6*</td>
</tr>
<tr>
<td>18:3</td>
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<td>0.4 ± 0.02</td>
<td>0.5 ± 0.05</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>20:4</td>
<td>384 ± 22</td>
<td>380 ± 25</td>
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</tr>
<tr>
<td>20:5</td>
<td>2 ± 0.3</td>
<td>1 ± 0.1</td>
<td>3 ± 0.5</td>
<td>2 ± 0.4</td>
</tr>
<tr>
<td>22:6</td>
<td>84 ± 5</td>
<td>76 ± 7</td>
<td>84 ± 3</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>Total</td>
<td>1377 ± 76</td>
<td>1366 ± 90</td>
<td>1313 ± 39</td>
<td>1356 ± 128</td>
</tr>
</tbody>
</table>
[00251] Data were expressed as means ± standard errors of the mean in wild-type mice fed a control diet (Wt Control, n=8), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=7), P301S mice fed a control diet (Tg Control, n=8), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=7). Bezafibrate treatment significantly decreased levels of poly-unsaturated fatty acids (Fisher PLSD, § Wt Control and Wt bezafibrate; † Wt Control and Tg Control; * Tg Control and Tg bezafibrate, p<0.05).

[00252] To have a better understanding of the impact of bezafibrate diet on brain lipid metabolism, a comprehensive lipidomic analysis of brain tissue derived from wild-type and P301S mice was also conducted. It was found that neither the P301S transgene expression, nor the bezafibrate diet caused any major changes in the levels of various lipid classes (Fig. 18A). However, two types of alterations were observed in the metabolism of sphingolipids: first, a significant increase in the levels of monohexyl ceramide (MHCer) was found in the P301S brains from mice treated with a control diet, but not with the bezafibrate diet; second, the wild-type mice on the bezafibrate diet showed an increase in the levels of sulfatide (Fig. 18A). Interestingly, while no major differences were found in the average carbon lengths and levels of unsaturated fatty acyl groups associated with glycerolipids and phospholipids (Fig. 18B,C), several alterations were noted in specific molecular species of various lipid families (Fig. 19). Of particular interest were brain lipid changes that correlate with the phenotypic rescue of P301S mice treated with the bezafibrate diet (see 4th lane in the heat map of Fig. 19). These include decreases in various ether phosphatidylcholine (ePC) species (ePC 34:0, 36:0, 36:1, 38:4), diacylglycerol (DG 34:2, 36:2, 36:3) and triglycerides (TG 52:2, 54:3, 58:9, 60:7, 60:9). The case of DG is particularly interesting as these very same species of DG (i.e., 34:2, 36:2, 36:3) are increased by expression of the P301S transgene (see 2nd lane in the heat map of Fig. 19), indicating that the bezafibrate diet reversed lipid alterations that occur in the brains of mice with the P301S tauopathy. Overall, these data indicate that the brain lipidome is differentially affected by expression of the P301S transgene, the bezafibrate diet and the two together.

Bezafibrate treatment had beneficial effects in brown adipose tissue of P301S mice

[00253] Body weight and lipid metabolism in brown adipose tissue were also studied (Fig. 15). Previous reports showed that bezafibrate reduces body weight (42). In the present study, both wild-type and P301S mice fed the bezafibrate diet had lower body
weights than wild-type and P301S mice fed the control diet (approximately a 30% reduction) (Fig. 15A,B). Interestingly, in the brown adipose tissue, P301S mice had an increase in the size and number of lipid vacuoles as compared to their wild-type littermates, using both hematoxylin and oil red O staining (Fig. 15C). This phenomenon was not due to the presence of human tau protein since the human tau protein was not detected using either immunohistochemistry or western blotting with the HT7 antibody (an anti-human tau antibody) (Fig. 21A-C). However, this pathology was improved after bezafibrate treatment, as evidenced by a reduction in size and number of lipid vacuoles in the brown adipose tissue of P301S mice, similar to the observations in the R6/2 transgenic mouse model of HD (29).

To determine how bezafibrate exerts beneficial effects in brown adipose tissue of P301S mice, gene expression of PPARs and their downstream targets involved in energy metabolism were measured as previously done in the brain, including Sirtl, Sirt3, uncoupling protein 1 (UCP1), PGClα, NRF1 and Tfam (Fig. 15D-F). It is noted that there was a trend towards a decrease of PGClα mRNA in the brown adipose tissue of P301S mice relative to their wild-type littermates, which may play a role in the lipid pathology. Bezafibrate administration increased both PPARα and PPARγ in the brown adipose tissue of P301S mice (Fig. 15D). Levels of Sirtl, PGClα, NRF1 and Tfam were significantly increased after bezafibrate treatment, suggesting an increase in energy metabolism.

**Bezafibrate treatment increased fatty acid β-oxidation in brown adipose tissue of P301S mice**

Expression of PPAR downstream target genes involved in fatty acid β-oxidation, including ACOX1, CPTIA, HMGCS2 were also measured (Fig. 15G). After bezafibrate treatment, the levels of ACOX1, CPTIA, HMGCS2 were significantly increased (Fig. 15G).

To investigate the effects of bezafibrate on free fatty acids in the brown adipose tissue of P301S mice, another cohort of mice was generated and 0.5% bezafibrate was administered by intraperitoneal injection in a single dose (Table 2). Mice were sacrificed 4 hours after the injection. Levels of 3 saturated fatty acids were decreased in P301S mice as compared to their wild-type littermates: docosanoic acid (C22:0), tetracosanoic acid (C24:0), and hexacosanoic acid (26:0). After bezafibrate treatment,
levels of saturated fatty acids were increased. In particular, levels of hexacosanoic acid were significantly increased in the P301S mice treated with bezafibrate (Table 2). Total levels of free fatty acids did not change after bezafibrate treatment. As in the brains, there was a decrease of all poly-unsaturated fatty acids in the brown adipose tissues of P301S mice injected with bezafibrate relative to P301S mice injected with a vehicle control (Table 2).

Table 2

Types and levels of free fatty acids in brown adipose tissues of P301S mice and their wild-type littermates with or without bezafibrate treatment

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Wt Control (n=4)</th>
<th>Wt Beza (n=4)</th>
<th>Tg Control (n=4)</th>
<th>Tg Beza (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>47 ± 4</td>
<td>41 ± 7</td>
<td>34 ± 7</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>14:0</td>
<td>1343 ± 147</td>
<td>1204 ± 267</td>
<td>934 ± 253</td>
<td>820 ± 219</td>
</tr>
<tr>
<td>16:0</td>
<td>9223 ± 942</td>
<td>6553 ± 730</td>
<td>7299 ± 1651</td>
<td>5291 ± 910</td>
</tr>
<tr>
<td>18:0</td>
<td>4363 ± 559</td>
<td>2827 ± 719</td>
<td>3756 ± 863</td>
<td>2345 ± 349</td>
</tr>
<tr>
<td>20:0</td>
<td>321 ± 61</td>
<td>232 ± 106</td>
<td>136 ± 38</td>
<td>148 ± 26</td>
</tr>
<tr>
<td>22:0</td>
<td>91 ± 13</td>
<td>75 ± 21</td>
<td>36 ± 9</td>
<td>49 ± 6</td>
</tr>
<tr>
<td>24:0</td>
<td>66 ± 8</td>
<td>57 ± 10</td>
<td>30 ± 4</td>
<td>39 ± 4</td>
</tr>
<tr>
<td>26:0</td>
<td>25 ± 2</td>
<td>27 ± 2</td>
<td>10 ± 0</td>
<td>20 ± 1*</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:1</td>
<td>55 ± 13</td>
<td>62 ± 20</td>
<td>31 ± 9</td>
<td>34 ± 12</td>
</tr>
<tr>
<td>16:1</td>
<td>2576 ± 372</td>
<td>2795 ± 771</td>
<td>1635 ± 386</td>
<td>1855 ± 604</td>
</tr>
<tr>
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<td>15176 ± 3091</td>
<td>14471 ± 4081</td>
<td>11030 ± 2646</td>
</tr>
<tr>
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<td>694 ± 209</td>
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<tr>
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<td>7 ± 1</td>
<td>5 ± 2</td>
<td>4 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Polyunsaturated</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>18:2</td>
<td>25886 ± 3911</td>
<td>17861 ± 5426</td>
<td>25740 ± 7641</td>
<td>12523 ± 2943</td>
</tr>
<tr>
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<td>1090 ± 137</td>
<td>839 ± 141</td>
<td>1204 ± 316</td>
<td>724 ± 135</td>
</tr>
<tr>
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<td>609 ± 115</td>
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<td>695 ± 218</td>
<td>302 ± 51</td>
</tr>
<tr>
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<td>101 ± 30</td>
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<td>102 ± 31</td>
<td>56 ± 11</td>
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<tr>
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<td>422 ± 73</td>
<td>282 ± 72</td>
<td>525 ± 159</td>
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<td>49384 ± 10466</td>
<td>57436 ± 15787</td>
<td>35952 ± 7556</td>
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[00257] Data were expressed as means ± standard errors of the mean in wild-type mice fed a control diet (Wt Control, n=4), wild-type mice fed a bezafibrate diet (Wt Bezafibrate, n=4), P301S mice fed a control diet (Tg Control, n=4), and P301S mice fed a bezafibrate diet (Tg Bezafibrate, n=4). Bezafibrate treatment significantly decreased levels
of poly-unsaturated fatty acids (Fisher PLSD, § Wt Control and Wt bezafibrate; † Wt Control and Tg Control; * Tg Control and Tg bezafibrate, p<0.05).

[00258] A comprehensive lipidomics analysis for the brown adipose tissue was conducted and it was found that two classes of phospholipids, phosphatidylinositol (PI) and phosphatidylserines (PS) were significantly downregulated in the P301S mice treated with bezafibrate, while there is a trend for an elevation of diacylglycerol (DG) in both wild-type and P301S animals treated with bezafibrate (Fig. 18D). A further examination of the levels of individual lipid species showed significant changes in the P301S animals treated with bezafibrate including decreases in multiple species of PI and PS containing polyunsaturated fatty acids, and increases of multiple species of DG and TG (3rd lane of heatmap on Fig. 20). A fatty acid analysis of the phospholipids showed that the acute bezafibrate treatment produced a significant increase in short chain phospholipids relative to longer chain phospholipids (Fig. 18E), as well as an increase in saturated and monosaturated phospholipids relative to polyunsaturated phospholipids.

DISCUSSION

[00259] There is a large body of evidence demonstrating the importance of PPARs in lipid metabolism, energy metabolism, and inflammation. Several groups have investigated the role of PPARs in the central nervous system, and PPARγ agonist effects, such as pioglitazone and rosiglitazone in models of neurodegenerative diseases (43, 44). It was previously shown that administration of pioglitazone extended survival and attenuated neuronal loss, gliosis, and oxidative damage in a transgenic mouse model of amyotrophic lateral sclerosis (ALS) (11), as did another group of investigators (12). Interestingly, neuroprotective effects were also found in transgenic mouse models of Alzheimer’s disease (AD) (45). PPARγ agonists reduced β-amloid levels and inflammation (6), as well as cerebrovascular dysfunction (8), and behavioral deficits in transgenic mouse models of AD (46). PPARγ agonists also enhance mitochondrial biogenesis (47).

[00260] Several clinical trials in AD patients have been initiated using PPARγ agonists. In one study, there was an increase in cerebral glucose metabolism after rosiglitazone treatment in the early stages of the disease (48). In both a pilot trial and a phase II clinical trial, the use of pioglitazone improved memory and cognition in AD patients who did not have apolipoprotein E4 alleles (49). However, in large phase III trials,
cognition was not significantly improved in AD patients (48, 50-52), suggesting that the mechanism of action of PPARγ agonists in animal models of amyloid deposition may differ from those in humans, or that therapeutic intervention once the AD pathology is fully developed may not be efficacious (53). Also, neurofibrillary tangles are not present in most of the animal models of amyloid deposition.

[00261] In fact, in AD and tauopathies, very little is known about the role of PPARs in relation to abnormalities in the tau protein. Tauopathies are a group of diseases in which the predominant pathology is neurofibrillary tangles and hyperphosphorylated tau, including fronto-temporal dementia, cortico-basal degeneration, and progressive supranuclear palsy, as well as AD. Tau pathology has been closely linked to the dementia which occurs in AD (21, 22). This pathology is also reflective of tau oligomer species which have been shown to be released in the interstitial space as well as cerebrospinal fluid (54). Tau oligomers appear to propagate and produce neurotoxic effects (55-57).

[00262] In addition to PPARγ, other PPAR agonists have also been tested as potential therapeutic agents for the treatment of neurodegenerative diseases. PPARα agonists, such as fenofibrate, showed promising effects in both MPTP and 6-OHDA mouse models of Parkinson’s disease (PD) (19). Here, the role of a pan-PPAR agonist on tau related pathology was investigated. Bezafibrate, a well-known PPAR agonist that has been used for more than 25 years in the treatment of elevated triglycerides and cholesterol, was chosen. Bezafibrate primarily activates PPARα, but also has some activity on both PPARβ and PPARγ (26, 58). Recently, Wenz and colleagues showed that all three isotypes of PPARs were elevated in muscle of mice treated with bezafibrate (59), while another study showed an increase in PPARα and PPARβ (60).

[00263] In the present study, transgenic mice with the P301S mutation, which causes fronto-temporal dementia in human patients (61-63) were used. These mice develop progressive tau pathology and neurodegeneration with early synaptic deficits, as well as inflammation (31). The P301S mice and their wild-type littermates were administered 0.5% bezafibrate (about 800 mg/kg/day) in the diet from 1 to 10 months of age. This is a relatively high dose to achieve the best possible brain levels to fully assess its effects. Pharmacodynamic analyses in rodents have been conducted with lower doses of bezafibrate, such as 10 to 100 mg/kg/day, the most clinically relevant dose being 10 mg/kg/day for patients (64-66). However, many groups studying the in vivo effects of
bezafibrate in mice used higher doses, such as 0.2% (320 mg/kg) or 0.5% (800 mg/kg) (59, 60, 67-69). Since fibrates cross the blood brain barrier slowly (70), to achieve therapeutic effects in the brain, 0.5% bezafibrate, which is the highest dose that had previously been shown to be tolerable and efficacious in mice (29, 59), was utilized.

[00264] After bezafibrate administration, immunoreactivity of both MCI, which immunostains non-filamentous cytoplasmic tau with a conformational change, and AT8, which immunostains phosphorylated tau, were decreased by 77% and 65% respectively in the brains of P301S mice. This demonstrates that bezafibrate reduces filamentous and non-filamentous tau pathology. To understand the mechanism by which bezafibrate improves tau pathology, its effects on GSK3β were studied. Phosphorylation levels of tau are regulated by a number of kinases and phosphatases (71, 72). However, several reports have shown that GSK3β is a specific therapeutic target for PPARs. Activation of PPARα by overexpression or treatment with fenofibrate inhibited the phosphorylation of GSK3β (73). Sharma et al. similarly demonstrated that activation of PPARγ in stable AML-12 hepatocyte cell lines downregulated GSK3β kinase activity (74). Even though no major changes were seen between P301S mice and their wild-type littermates at baseline, it was found that bezafibrate significantly reduced phospho-GSK3P levels.

[00265] In addition to tau pathology, P301S mice show early activation of microglia (31). PPAR agonists are well known to reduce inflammation by inhibiting the transcriptional regulation of pro-inflammatory genes such as the NFκB pathway (75), therefore, microglia activation was studied after bezafibrate treatment. CD11b immunoreactivity, which is a marker for activated microglia, was significantly reduced by bezafibrate treatment in the brains of P301S mice. Levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2), two critical mediators of inflammation, and are repressed by PPAR activation, were assessed. Bezafibrate treatment inhibited both iNOS and COX2 expression in the brains of P301S mice. In a model of neuro-inflammation produced by intracerebral injection of lipopolysaccharide in mice, pharmacological activation of PPARα decreased expression of tumor necrosis factor α (TNFa), COX2, and iNOS as well as other markers of inflammation (76). A PPARβ/δ agonist also decreased iNOS after septic shock (77). In transgenic AD mice, pioglitazone reduced glial inflammation by decreasing levels of iNOS and COX2 (6). Recently, Escribano and colleagues found that rosiglitazone inhibited microglial activation by
decreasing expression of TNFa and COX2 in transgenic AD mice. Consistent with these data, rosiglitazone also elevated the expression of the gene cluster of differentiation 36 (CD36) (24). The authors concluded that the PPARγ agonist rosiglitazone induced a switch of microglia activation from the classic (M1) to the alternative phenotype (M2), which could contribute to a reduction of tau accumulation in transgenic AD mice (24).

The beneficial effects of bezafibrate in P301S mice were associated with an improvement in behavioral deficits at various ages. P301S mice are known to be hyperactive and disinhibited as compared to wild-type mice (32, 33). In this study, bezafibrate treatment improved both hyperactivity and disinhibition at 5, 7, and 9 months of age. Chronic administration of rosiglitazone prevents early cognitive impairment in young transgenic AD mice, and reversed memory decline in aged transgenic AD mice, as shown using the object recognition test (24, 46). Further investigation to determine whether bezafibrate has effects on cognition could be conducted.

To confirm that PPAR activation occurred in the brains of the mouse model, gene expression levels of the three PPAR isotypes (PPARα, PPARβ, and PPARγ), and their downstream targets were assessed. In bezafibrate treated P301S mice, there was an increase of both PPARα and PPARβ in the brain. Consistent with PPARα activation being involved in fatty acid (FA) β-oxidation (78, 79), it was found that bezafibrate treatment increased gene expression of 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2 (HMGCS2). This effect was associated with a reduction of poly-unsaturated free fatty acid levels in the brains of P301S mice. These data are consistent with a previous report showing that in rats, levels of mitochondrial HMG-CoA synthase (mHS), ACOX, and medium chain acyl CoA dehydrogenase (MCAD) were increased after ciprofibrate administration (80).

To further examine the effects of bezafibrate on brain lipid metabolism, a comprehensive lipidomic analysis was conducted. Unlike the dramatic changes in subclass lipid composition present in familial AD mouse models (81), only two alterations of sphingolipids with increased levels of MHCer were observed in the untreated P301S mice, and sulfatide was observed in wild-type mice in the brain, following treatment with bezafibrate. However, numerous other lipid species were differentially regulated, the most interesting being an elevation of various DG species in the P301S mice followed by its reduction with bezafibrate treatment. This could have important implications since
accumulation of DG, a major bioactive lipid, may lead to overactivation of PKC, a biochemical signaling pathway that impairs working memory in rats (82).

[00269] The effects of bezafibrate on energy metabolism and oxidative stress were also examined. Indeed, PPARs are known to play a role in the induction of mitochondrial biogenesis via the peroxisome proliferator-activated receptor gamma coactivator 1-a (PGC1α) pathway (83, 84). Bezafibrate treatment decreased oxidative stress as shown by decreased levels of both protein carbonyls and GSSG/GSH in P301S mice. This effect of bezafibrate was not due to an increase in activity of mitochondrial electron transport enzymes as they remained unchanged after bezafibrate treatment. Interestingly, bezafibrate augmented gene expression of the mitochondrial transcription factor A (Tfam) in the brains of P301S mice, which was accompanied by an increase of mtDNA copy number. Although no major changes were observed between P301S mice and their wild-type littermates regarding energy metabolism, it is possible that some of the beneficial effects of bezafibrate may be secondarily mediated by increased mitochondrial biogenesis, which leads to a reduction of oxidative stress (85). This finding suggests that bezafibrate improved pathways that did not appear damaged in non-treated P301S mice.

[00270] It was found that body weights of mice treated with the bezafibrate diet were markedly reduced. Even though tau pathology is found mostly in the brain in the P301S mice, the effect of bezafibrate in brown adipose tissue were studied, since it is a tissue which develops marked increased of lipid vacuoles in response to a reduction in PGC1α. Brown adipose tissue, which is used to generate body heat in mammals, contains small lipid droplets also called lipid vacuoles and large numbers of mitochondria (86).

[00271] In the study, long-term bezafibrate treatment improved brown adipose tissue pathology in P301S mice, by reducing the size and number of lipid droplets. To better understand these beneficial effects, expression of PPARs and their downstream targets in the brown adipose tissue of P301S mice was examined. Both PPARα and PPARγ mRNA levels were increased in the brown adipose tissue. In isolated adipocytes, activation of PPARα with bezafibrate reduces adipocyte hypertrophy and elevates both adipogenic and FA oxidation related genes, such as peroxisomal acyl-coenzyme A oxidase (ACOX), and carnitine-palmitoyl transferase 1 (CPT1) (87), similar to the observations in the P301S mice. Using a chromatin immunoprecipitation (ChIP) assay, bezafibrate treatment resulted in the recruitment of PPARα to the promoter regions of both adipogenic
and FA oxidation related genes (87). In a mouse model of mitochondrial myopathy, bezafibrate treatment induced FA oxidation in the liver, as evidenced by increases of both the FA translocase CD36, and ACOX1 (69). Similar effects occurred in the muscle of mice treated with bezafibrate (60).

Since a reduction in lipid droplets was observed, it suggests that bezafibrate treatment may induce β-oxidation of fatty acids and lipidoxidation of triglyceride, the major lipid component of brown adipose tissue, and/or other lipid classes. To investigate this possibility, the lipidome of brown adipose tissue was examined after acute bezafibrate treatment by intraperitoneal injections. Similar to the brains of P301S mice undergoing long-term treatment of bezafibrate, levels of poly-unsaturated free fatty acids were reduced in acutely treated mice. This data is consistent with previous data published by Tremblay-Mercier et al. (2010) showing that bezafibrate lowered plasma levels of free fatty acids in patients with hypertriglyceridemia as a result of increased FA β-oxidation of linoleic acid (88). In the present study, further examination of the lipidome revealed that only PS and PI were significantly decreased in P301S mice treated with bezafibrate.

However, there was a clear shift towards phospholipids with shorter fatty acid chain length and a lower degree of saturation, thereby suggesting that significant lipid remodeling is taking place due to bezafibrate treatment. Interestingly, there was also a trend for an increase in DG in bezafibrate-treated mice, suggesting potential activation of lipolysis of TG, even within this small time window.

In the brown adipose tissue, beneficial effects of bezafibrate in P301S mice may also be attributed to its ability to increase expression of energy metabolism genes and mitochondrial biogenesis, such as PGClα, NRF1, Tfam and Sirt 1. These effects of PPAR activation on transcription factors which regulate mitochondrial biogenesis were also observed in other models, such as in the muscle of bezafibrate treated mice with a mitochondrial myopathy (89), and in the brains of mice treated with rosiglitazone (47). It was also found that bezafibrate increased brain and muscle mitochondrial biogenesis in the R6/2 transgenic mouse model of HD (29). However, in studies of other mice with different myopathies, bezafibrate treatment had no effect on mitochondrial biogenesis (60, 69). The effects of bezafibrate on mitochondrial biogenesis therefore appear to vary with the background strains of the mice being studied, or with the origin of the mitochondrial dysfunction or its metabolic context.
In conclusion, it was shown that bezafibrate exerts beneficial effects in both the central nervous system as well as brown adipose tissue in the P301S transgenic mice. This is the first *in vivo* evidence showing that bezafibrate ameliorates tau pathology and behavioral deficits in diseases that manifest tauopathy. The beneficial effects of bezafibrate are associated with its ability to reduce inflammation and stimulate lipid metabolism. Importantly, bezafibrate was administered prior to the appearance of any tau pathology, which supports a possible preventive role of bezafibrate. The present results therefore show that drugs such as bezafibrate could be useful for the treatment of patients with tauopathies or other diseases such as AD, in which tau pathology plays a major role, both administered during symptoms but especially when given during pre-symptomatic stages of the disease.

**MATERIALS AND METHODS**

*Animals and treatment*

Animals were generated by breeding P301S transgenic male mice with wild-type female mice, under the original C57BL/6 x C3H background obtained from Jackson Laboratory (Bar Harbor, ME, USA). Offspring were genotyped by PCR of tail DNA. P301S transgenic mice and their wild-type littermates were randomly assigned to receive either control diet (LabDiet 5002) or 0.5% bezafibrate diet (Sigma, St. Louis, MO, USA) from 1 to 10 months of age ad libitum. The chow was pelleted by Purina-Mills (Richmond, IN, USA).

Behavioral analyses were performed at 5, 7, and 9 months of age. Histopathological and biochemical analyses were conducted at 10 months of age on the same animals. All experiments were approved by the Weill Cornell Medical College Institutional Animal Care and Use Committee.

*Behavior*

Body weight was recorded once a month from 2 to 9 months of age. Locomotor activity and exploration were assessed in the open field test as previously described (90). Briefly, mice were placed in the apparatus for a 5 min trial. Distance traveled and rearing frequency were recorded using a video tracking system (Ethovision 3.1, Noldus Technology, Attleborough, MA, USA). As an indicator of exploration and anxiety, the time spent in the periphery and the center of the apparatus was recorded.
After behavioral testing, half of the mice in each group were sacrificed by decapitation. Brains were collected, dissected, snap frozen in liquid nitrogen, and stored at -80 °C for biochemical studies.

Tissues were homogenized in RIPA buffer with protease and phosphatase inhibitors (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Equal amounts of protein were electrophoresed through 4-12% Tri-Bis NuPage gels (Invitrogen, Carlsbad, CA, USA). After transfer to polyvinylidene fluoride (PVDF), membranes were blocked in 5% non-fat dry milk in phosphate buffer saline with 0.05% Tween 20 (PBST) and exposed overnight to the primary antibody at 4 °C. Horseradish peroxidase-conjugated (HRP) secondary antibody binding was visualized with enhanced chemiluminescence (Pierce, Rockford, IL, USA).

Primary antibodies and concentrations used for western blotting were: mouse monoclonal anti-GSK3P (1:500, Abeam, Cambridge, MA, USA); rabbit monoclonal anti-phospho-GSK3β (Y216) (1:1,000, Cell Signaling, Danvers, MA, USA); rabbit polyclonal anti-COX2 (1:200, Abeam, Cambridge, MA, USA); rabbit polyclonal anti-iNOS (1:500, Santa Cruz, Biotechnology, Santa Cruz, CA, USA); and mouse monoclonal anti-β-actin (1:10,000, Sigma, St. Louis, MO, USA). Quantitative analysis was performed using NIH-based Scion Image software (Scion Corp., Frederick, MD, USA). Statistical analysis was performed using ratios of the densitometric value of each band normalized to β-actin as loading control.

Immunohistochemistry and histology

The remaining mice from each group were deeply anesthetized using sodium pentobarbital and transcardially perfused with ice-cold 0.9% sodium chloride and 4% paraformaldehyde. Brains were collected, dissected, post-fixed in 4% paraformaldehyde followed by gradient sucrose (15% and 30%), and stored in cryoprotectant for immunohistochemical studies.

Sections were cut at 50 µm thickness and stained with the following antibodies: MCI mouse monoclonal anti-human tau (N-terminal conformational change, Exon 10) (1:500, gift from Dr. Peter Davies); AT8 mouse monoclonal anti-human tau pSer202/Thr205 (1:500, Thermo Fisher Scientific, Rockford, IL, USA); rat monoclonal
anti-CD11b (1:1,000, AbD Serotec, Raleigh, NC, USA). Immunolabeling was detected by
the streptavidin-horseradish peroxidase method and visualized after diaminobenzidine
(DAB) incubation (Vector, Burlingame, CA, USA). Quantification was done using five 50
µm serial non-adjacent sections per animal (300 µm apart, from bregma -1.34 through
bregma -2.84 through the cerebral cortex and hippocampus). The percentage area
occupied by AT8 and the intensity (optical density) of CD11b were measured using Scion
Image (Scion Corp., Frederick, MD, USA). For the cerebral cortex, measures were
determined within a 0.9 mm² area encompassing the primary (M1) and secondary (M2)
motor cortex. The threshold was set at 140. For the hippocampus, the percent area
occupied by AT8-labeled structures in the CA1 was determined. The intensity of CD11b-
stained microglia in the hippocampus was measured within a 0.9 mm² area using the
dentate gyrus as an anatomical landmark. The threshold was set at 140. The number of
neurons intensely stained with MCI were counted using Scion Image (Scion Corp.,
Frederick, MD, USA). There were few MCI immunoreactive neurons in both the cerebral
cortex and hippocampus. For this reason, stereological analysis was not used because this
approach requires a higher number of countable cells by applying the random paradigm.
For the cerebral cortex, the numbers of MCI-positive neurons were counted within a 0.9
mm² area encompassing the primary (M1) and secondary (M2) motor cortex. For
consistency, the cingulum was used as a landmark. For the hippocampus, the numbers of
MCI-positive neurons were counted in the CA1 region of the hippocampus. Results are
expressed as mean of the numbers of MCI-positive neurons/section.

[00283] For the histology, perfused and fixed brown adipose tissues were cut at 16
µm thickness and mounted on slides. Sections were processed for hematoxylin-eosin and
oil red O staining to visualize lipid vacuoles in the brown adipose.

Gene expression by qRT-PCR

[00284] Fresh frozen tissues stored at -80 °C were processed for RNA extraction
(Qiagen kit, Valencia, CA, USA). Quantitative real-time PCR (qRT-PCR) was performed
at the Weill Cornell Medical College Microarray Core Facility using SyberGreen assays
with the ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City,
CA, USA). The following genes were analyzed: PPARa, PPARβ, PPARy, GSK3β,
ACOX1, CPT1A, HMGCS2, PGCla, NRF1, Tfam, UCP1, Sirt 1, Sirt 3, COX2, iNOS
and GAPDH as control.
Mitochondria characterization - Sample preparation. Dissected, non-perfused frontal lobe samples (-30-55 mg) were stored frozen at -80 °C until assaying. Before assays, tissue samples were thawed on ice and homogenized with Dounce-type 2 ml homogenizer (glass vessel/glass pestle). The homogenate was centrifuged at 1,000 g x 5 min to get rid of nuclear fraction and cell debris; the resulting supernatant was centrifuged at 14,000 g x 5 min. The pellet was collected and centrifuged again at 14,000 g x 5 min; the final pellet obtained in this step was resuspended in 20 mM HEPES (pH 7.8) and used for all assays.

Immunoblot Analysis - mitochondria-enriched fraction. The protein lysates containing equal amounts of protein were separated by SDS-PAGE, electroblotted onto a nitrocellulose membrane (BioRad, Hercules, CA, USA), and immunoreacted with an appropriate primary antibody (see below), followed by HRP-conjugated secondary antibodies (Kierkegaard Perry Labs Inc., Gaithersburg, MD, USA). Immunoreactive proteins were visualized by incubating blots in chemiluminescence substrate (Pierce, Rockford, IL, USA). Quantitative analysis was performed using NIH "Image J" software. Statistical analysis was performed using ratios of the densitometric value of each band normalized to β-actin as loading control.

Assays. All samples were assayed for the following: complex I activity (NADH:CoQ reductase, rotenone-sensitive (91)), malic enzyme activity (malate:NADP reductase), isocitric dehydrogenase activity (threo-Ds-isocitrate:NADP+ reductase), succinate dehydrogenase activity (succinate:CoQ:DCIP reductase, TTFA-sensitive (92)), citrate synthase activity (93), aconitase activity ("Aconitase Assay Kit", Cayman Chemical, MI, USA), glutathione reductase activity ("Glutathione Reductase Assay Kit", Cayman Chemical, Ann Arbor, MI, USA), superoxide dismutase activity ("Superoxide Dismutase Assay Kit", Cayman Chemical, Ann Arbor, MI, USA), ATPase subunit expression (immunoblotting with anti-ATPase ATP5A1 subunit 1:1,000, Invitrogen, Carlsbad, CA, USA), and protein carbonyls by DNP derivatization followed by immunoblotting ("Oxyblot" kit, Cayman Chemical, Ann Arbor, MI, USA). All activities and content values were normalized by protein content (BCA protein assay, Thermo Scientific, FL, USA).
mtDNA copy number

Frozen tissues stored at -80 °C were processed for DNA extraction according to the manufacturer's protocol (Qiagen kit, Valencia, CA, USA). The relative mtDNA copy number was determined by quantitative real-time PCR on an ABI PRISM 7900H Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the TaqMan® Universal PCR Master mix and predeveloped TaqMan® Gene Expression Assay primers/probes (Applied Biosystems, Foster City, CA, USA) for mitochondrial cytochrome oxidase 2 and β-actin (nuclear DNA control). Results were calculated from the threshold cycle values and expressed as the 2^ΔCT of cytochrome oxidase 2 to β-actin.

Lipidomics-lipid extraction

Lipid extraction was performed using a modified Bligh and Dyer protocol and spiked with an internal standard mixture as described previously (81). Mass spectrometry analyses were done with an Agilent Technologies 6490 Ion Funnel LC/MS Triple Quadrupole system with front end 1260 Infinity HPLC. Phospholipids and sphingolipids were analyzed by normal phase HPLC while neutral lipids were analyzed using a reverse phase HPLC. For normal phase analysis, lipids were separated on a Phenomenex Luna silica column (i.d. 2.0 x 150 mm) using a gradient consisting of A: chloroform/ methanol/ ammonium hydroxide (90:9.5:0.5) and B: chloroform/ methanol/ water/ ammonium hydroxide (55:39:5.5:0.5), starting at 5% and changing to 70% over a 40min period as described previously (94). Neutral lipids were separated on an Agilent Zorbax XDB-C18 column (i.d. 4.6x100 mm) using an isocratic mobile phase chloroform: methanol: 0.1M ammonium acetate (100:100:4) at a flow rate of 30μmin (94, 95). The instrument capillary voltage, sheath gas flow rate and temperature and nebulizer pressure was set to 3000V, 12L/min, 300°C and 35 psi respectively. Multiple reaction monitoring (MRM) transitions were set-up for quantitative analysis of different lipid subclasses as described previously (81). The solvents employed for sample extractions and liquid chromatography were LC/MS grade or LC grade when LC/MS grade was not available and were purchased from Sigma.

Freefatty acid levels

Unesterified fatty acid concentrations were determined for brain and brown adipose samples using negative ESI-MS and the selected ion recording (SIR) mode
exactly as described by Clugston et al. (96). All measurements were carried out on a Waters Xevo TQ MS ACQUITY UPLC system (Waters, Milford, MA, USA). The system was controlled by Mass Lynx Software version 4.1. (Waters, Milford, MA, USA). The solvents employed for sample extractions and liquid chromatography were LC/MS grade or LC grade when LC/MS grade was not available and were purchased from Thermo Fisher (Pittsburgh, PA, USA).

Oxidative stress markers (HPLC)

[00291] Malondialdehyde levels. The HPLC determination of MDA was carried out by a method modified from a previous report by Agarwal and Chase in 2002 (97). Fresh tissues were homogenized in 40% ethanol solution. 50 µl of sample homogenate or MDA standard were prepared in 40% ethanol. 50 µl of 0.05% butylated hydroxytoluene (BHT), 400 µl of 0.44 M H3PO4, and 100 µl of 0.42 mM 2-thiobarbituric acid (TBA) were added to each. Samples were then vortexed, heated for 1 hr at 100 °C, and immediately cooled with iced water to stop the derivative reaction. The MDA-TBA derivative was extracted by adding 250 µl n-butanol, followed by vortexing and centrifugation. 50 µl of n-butanol extract was used for the HPLC assay. The HPLC mobile phase used acetonitrile buffer (20:80, v/v, buffer 50 mM KH2PO4, pH 6.8). The column was an ESA 150 x 3 mm C18 column with particle size of 3 µm (ESA, Inc., Bedford, MA, USA). Fluorescence detectors were set at an excitation wavelength of 515 nm and emission wavelength of 553 nm. MDA was eluted from the column in 2 min. Data were normalized by protein content (Biorad Protein Assay Kit, Hercules, CA, USA).

[00292] GSH and GSSG levels. Brain tissues were homogenized and centrifuged in chilled 0.1 M perchloric acid. Supernatants were used for reduced (GSH) and oxidized (GSSG) glutathione measurements by HPLC, as previously described (98). Briefly, 15 µl supernatant was isocratically eluted through a 4.6 x 150 mm C18 column (ESA, Inc Chelmsford, MA, USA) with a mobile phase containing 50 mM LiH2PO4, 1.0 mM 1-octanesulfonic acid and 1.5% (v/v) methanol. The 2-channel Coulochem III electrochemical detector (ESA, Inc. Chelmsford, MA, USA) was set with guard cell potential 950 mV, Channel 1 potential 500 mV for GSH detection and Channel 2 potential 880 mV for GSSG detection. Concentrations of both GSH and GSSG are expressed as nmol per milligram of protein. Protein concentrations of tissue homogenates were
measured using the Bio-Rad protein protocol (Bio-Rad Laboratories, Hercules, CA, USA) and Perkin Elmer Bio Assay Reader (Norwalk, CT, USA).

Statistical analysis

For behavioral data, ANOVA with repeated measurements was used followed by Post-hoc Fisher's PLSD tests for multiple comparisons (comparing four groups: wild-type mice fed control diet, wild-type mice fed bezafibrate diet, P301S mice fed control diet and P301S mice fed bezafibrate diet). To analyze any other data, two-tailed unpaired t-tests (comparing two groups: P301S mice fed control diet and P301S mice fed bezafibrate diet), and post-hoc Fisher's PLSD tests for multiple comparisons (comparing four groups: wild-type mice fed control diet, wild-type mice fed bezafibrate diet, P301S mice fed control diet and P301S mice fed bezafibrate diet) were used (Statview 5.0.1, SAS Institute Inc., Cary, NC, USA). All presented data were expressed as means ± standard errors of the means.

REFERENCES:


1. A method for prevention and treatment of a neurodegenerative disease comprising administering to a subject in need a therapeutic composition comprising a prevention or treatment effective amount of a pan-PPAR agonist.

2. The method of claim 1, wherein the pan-PPAR agonist is bezafibrate.

3. The method of claim 1, wherein the neurodegenerative disease is Huntington's disease.

4. The method of claim 1, wherein the neurodegenerative disease is a tauopathy or Alzheimer's Disease.

5. The method of claim 4, wherein the tauopathy is a member of Pick's complex.

6. The method of claim 5, wherein the member of Pick's complex is Progressive supranuclear palsy; Dementia pugilistica (chronic traumatic encephalopathy); traumatic encephalopathy; Frontotemporal dementia and parkinsonism linked to chromosome 17; Lytico-Bodig disease (Parkinson-dementia complex of Guam); Tangle-predominant dementia; Ganglioglioma; gangliocytoma; Meningioangiomatosis; Subacute sclerosing panencephalitis; lead encephalopathy; tuberous sclerosis; Hallervorden-Spatz disease; lipofuscinosis; Pick's disease; corticobasal degeneration; Argyrophilic grain disease (AGD); corticobasal degeneration; Frontotemporal dementia; or Frontotemporal lobar degeneration.

7. The method of claim 1, wherein the therapeutic composition further comprises a pharmaceutically acceptable carrier or excipient for a suitable formulation and administration.

8. The method of claim 7, wherein the therapeutic composition is administered orally.

9. The method of claim 7, wherein the therapeutic composition is administered intravenously, intramuscularly, or via a suitable parental administration route.

10. The method of claim 7, wherein the therapeutic composition is formulated for controlled-release.

11. The method of claim 7, wherein the therapeutic composition is formulated for sustained-release.

12. The method of claim 1, wherein the therapeutic composition further comprises a second pan-PPAR agonist selective to another PPAR receptor subtype.
13. The method of claim 1, wherein the therapeutic composition is administered in conjunction with other therapeutic agent before, during, or after the administration of the pan-PPAR agonist.


15. The composition of claim 14, wherein the pan-PPAR agonist is bezafibrate.

16. The composition of claim 14, wherein the neurodegenerative disease is Huntington’s Disease.

17. The composition of claim 14, wherein the neurodegenerative disease is a tauopathy or Alzheimer's Disease.

18. The composition of claim 17, wherein the tauopathy is a member of Pick's complex.

19. The composition of claim 18, wherein the member of Pick's complex is Progressive supranuclear palsy; Dementia pugilistica (chronic traumatic encephalopathy); traumatic encephalopathy; Frontotemporal dementia and parkinsonism linked to chromosome 17; Lytico-Bodig disease (Parkinson-dementia complex of Guam); Tangle-predominant dementia; Ganglioglioma; gangliocytoma; Meningioangiomatosis; Subacute sclerosing panencephalitis; lead encephalopathy; tuberous sclerosis; Hallervorden-Spatz disease; lipofuscinosis; Pick's disease; corticobasal degeneration; Argyrophilic grain disease (AGD); or corticob.

20. The composition of claim 14, further comprising a pharmaceutically acceptable carrier and formulated for oral administration, parental administration, immediate-release, controlled-release, or sustained-release.
FIGURE 3A

Mitochondrial Density

<table>
<thead>
<tr>
<th>Treatment/Gene Type</th>
<th>Mitochondrial Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt. standard</td>
<td>7.12</td>
</tr>
<tr>
<td>wt. bezafibrate</td>
<td>8.62</td>
</tr>
<tr>
<td>R6/2. standard</td>
<td>3.66</td>
</tr>
<tr>
<td>R6/2. bezafibrate</td>
<td>1.36</td>
</tr>
</tbody>
</table>

FIGURE 3C

Figure 3D

SUBSTITUTE SHEET (RULE 26)
Malondialdehyde immunoreactivity in Striatum

FIGURE 5A

Malondialdehyde measurement by HPLC

FIGURE 5B
Succinate dehydrogenase histochemistry of soleus muscle

FIGURE 6A

Quantitation of SDH histochemistry of soleus muscle

FIGURE 6B
Electron micrograph of soleus muscle

FIGURE 6C
FIGURE 8
AT8 immunostaining

FIGURE 10C

AT8 quantification

FIGURE 10D
FIGURE 12G

COX2 protein

Tg Bezafibrate
WT Control
WT Bezafibrate
Tg Control

FIGURE 12H

COX2/β-actin

O.D./mg of protein

WT Control
WT Bezafibrate
Tg Control
Tg Bezafibrate

H E O
Open field: Distance moved

- Wt Control (n=16)
- Wt Bezafibrate (n=16)
- Tg Control (n=14)
- Tg Bezafibrate (n=13)

Distance (cm)

Age (months)

FIGURE 13A

Open field: Rearings

Number of rearings

Age (months)

FIGURE 13B
Open field: Anxiety

Time spent in center (sec)

† † †

5 7 9

Age (months)

FIGURE 13C
Figures 15F and 15G depict the fold change in gene expression for Metabolism mRNA and FA oxidation mRNA, respectively. The graphs show the expression levels of different genes under various conditions: Wt Control, Wt Bezafibrate, Tg Control, and Tg Bezafibrate. The y-axis represents the fold change, and the x-axis lists the genes Sirt1, Sirt3, UCP1, HMGC82, CPT1A, and ACOX1.
Antioxidant enzyme activity

![Graph showing antioxidant enzyme activity for Glutathione reductase and Superoxide dismutase.]

**FIGURE 17A**

Mitochondrial enzyme activity

![Graph showing mitochondrial enzyme activity for various enzymes including Aconitase, Citrate synthase, Complex I, Succinate dehydrogenase, Isocitrate dehydrogenase, and Malic enzyme.]

**FIGURE 17B**
ATPase protein

![Bar chart showing ATPase protein with error bars]

FIGURE 17C
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/216(2006.01)i, A61K 31/215(2006.01)1, A61P 25/28(2006.01)1, A61P 25/18(2006.01)1, A61P 25/00(2006.01)1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & keywords: PPAR agonist, peroxisome proliferator-activated receptor, bezafibrate, Alzheimer's disease, Huntington's disease

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X/A</td>
<td>US 2002-0055529 A1 (BISGAIER, C. L. et al.) 9 May 2002 See claims 1, 3.</td>
<td>14,15,17,20/16,18,19</td>
</tr>
<tr>
<td>X/A</td>
<td>US 2009-0143279 A1 (MOOTHA, V. K. et al.) 4 June 2009 See claims 1, 25, 39.</td>
<td>14-17,20/18,19</td>
</tr>
</tbody>
</table>

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier publication or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed
  "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  "&" document member of the same patent family

Date of the actual completion of the international search
13 MARCH 2013 (13.03.2013)

Date of mailing of the international search report
14 MARCH 2013 (14.03.2013)

Name and mailing address of the ISA/KR

Facsimile No. 82-42-472-7140

Authorized officer
HAN, In Ho
Telephone No. 82-42-481-3362

Form PCT/ISA/210 (second sheet) (July 2009)
INTERNATIONAL SEARCH REPORT

PCT/US2012/064410

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: 1-13
   because they relate to subject matter not required to be searched by this Authority, namely:
   Claims 1-13 pertain to a method for treatment of the human by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.
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