

Acute treatment with the PPAR γ agonist pioglitazone and ibuprofen reduces glial inflammation and A β 1–42 levels in APPV717I transgenic mice

Michael T. Heneka,¹ Magdalena Sastre,¹ Lucia Dumitrescu-Ozimek,¹ Anne Hanke,¹ Ilse Dewachter,² Cuno Kuiperi,² Kerry O'Banion,³ Thomas Klockgether,¹ Fred Van Leuven² and Gary E. Landreth⁴

¹Department of Neurology, University of Bonn, Bonn, Germany, ²Experimental Genetics Group, Department of Human Genetics, K.U. Leuven, Leuven, Belgium, ³Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY and ⁴Department of Neurosciences, Alzheimer Research Laboratory, Case Western Reserve University, Cleveland, OH, USA

Correspondence to: Michael T. Heneka, Department of Neurology, University of Münster, Albert-Schweitzer-Str. 33, Münster, Germany
E-mail: heneka@uni-muenster.de

Summary

Neuritic plaques in the brain of Alzheimer's disease patients are characterized by β -amyloid deposits associated with a glia-mediated inflammatory response. Non-steroidal anti-inflammatory drug (NSAID) therapy reduces Alzheimer's disease risk and ameliorates microglial reactivity in Alzheimer's disease brains; however, the molecular mechanisms subserving this effect are not yet clear. Since several NSAIDs bind to and activate the nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR γ) which acts to inhibit the expression of proinflammatory genes, this receptor appears a good candidate to mediate the observed anti-inflammatory effects. Recent data *in vitro* suggested that NSAIDs negatively regulate microglial activation and immunostimulated amyloid precursor protein processing via PPAR γ activation. We report that an acute 7 day oral treatment of 10-month-old APPV717I mice with the PPAR γ agonist

pioglitazone or the NSAID ibuprofen resulted in a reduction in the number of activated microglia and reactive astrocytes in the hippocampus and cortex. Drug treatment reduced the expression of the proinflammatory enzymes cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS). In parallel to the suppression of inflammatory markers, pioglitazone and ibuprofen treatment decreased β -secretase-1 (BACE1) mRNA and protein levels. Importantly, we observed a significant reduction of the total area and staining intensity of A β 1–42-positive amyloid deposits in the hippocampus and cortex. Additionally, animals treated with pioglitazone revealed a 27% reduction in the levels of soluble A β 1–42 peptide. These findings demonstrate that anti-inflammatory drugs can act rapidly to inhibit inflammatory responses in the brain and negatively modulate amyloidogenesis.

Keywords: Alzheimer's disease; PPAR; inflammation; NSAID; neurodegeneration

Abbreviations: A β = amyloid β ; APP = amyloid precursor protein; COX = cyclooxygenase; BACE-1 = β -secretase-1; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; GFAP = glial fibrillary acidic protein; IB4 = isolectin B4; iNOS = inducible nitric oxide synthase; NSAID = non-steroidal anti-inflammatory drug; PPAR γ = peroxisome proliferator-activated receptor- γ ; PCR = polymerase chain reaction

Received August 31, 2004. Revised October 19, 2004. Accepted January 21, 2005. Advance Access publication April 7, 2005

Introduction

Inflammation plays an important role in the pathogenesis of a number of neurodegenerative diseases and arises as a consequence of the primary disease process. Alzheimer's disease is clinically characterized by progressive memory loss and decline of cognitive functions. Its classical histopathological hallmarks include extracellular amyloid peptide (A β) deposition in neuritic plaques, and intracellular deposits of hyperphosphorylated tau, causing formation of neurofibrillary tangles and finally neuronal death. A β peptides are generated from the amyloid precursor protein (APP) by sequential actions of two proteolytic enzymes, the β -secretase 1 (BACE1) and the γ -secretase (Dingwall, 2001; Esler and Wolfe, 2001). Formation and aggregation of A β represent a key feature and possibly a triggering mechanism of Alzheimer's disease. The importance of A β formation is supported by dominantly inherited familial forms of Alzheimer's disease that are linked to APP mutations in or close to the β - and γ -secretase cleavage sites (Hardy and Allsop, 1991). This made it possible to generate transgenic mouse models of cerebral amyloidosis and Alzheimer-like histopathology, i.e. amyloid plaques and cerebral amyloid angiopathy (Hsiao *et al.*, 1995; Sturchler-Pierrat *et al.*, 1997; Lamb *et al.*, 1999; Moechars *et al.*, 1999; Van Dorpe *et al.*, 2000).

Next to neurodegeneration, Alzheimer's disease shows a significant inflammatory component that is characterized by the presence of abundant and activated glial cells associated with fibrillar deposits of A β that comprise the senile plaque (Akiyama *et al.*, 2000; Wyss-Coray and Mucke, 2002). Once initiated, inflammatory processes may contribute independently to neural dysfunction and cell death, thereby establishing a self-perpetuating vicious cycle (Griffin *et al.*, 1998), by which the inflammation may represent the primary cause of further neurodegeneration.

In the CNS, the cellular mediators of the innate immune response are the microglia cells whose activation results in the production of a diverse range of inflammatory molecules. Once activated, microglia release a diverse range of cytokines and other proinflammatory products, which contribute to neurodegeneration and exacerbation of the inflammatory reaction by recruiting and stimulating astrocytes. The recognition that a robust inflammatory response is a component of the disease process led to the discovery that long-term treatment with non-steroidal anti-inflammatory drugs (NSAIDs) dramatically reduced the risk for Alzheimer's disease, delayed disease onset, ameliorated symptomatic severity and slowed cognitive decline (McGeer *et al.*, 1996; Stewart *et al.*, 1997; In t'Veld *et al.*, 2001). In addition, NSAID treatment was positively correlated with a reduction of plaque-associated, activated microglia in humans (Mackenzie and Munoz, 1998; Alafuzoff *et al.*, 2000). This observation is supported by two recent studies which demonstrate that the NSAID ibuprofen acts to reduce microglial activation and cytokine production in transgenic mice overexpressing APP (Lim *et al.*, 2000; Yan *et al.*, 2003). Importantly,

a 6 month oral ibuprofen treatment also reduced the amount of A β deposited in the brains of these animals.

The mechanisms by which NSAIDs act to reduce inflammation in Alzheimer's disease are controversial. The established targets of NSAIDs are cyclooxygenases (COXs) 1 and 2, and experimental data using a neuronal COX2 overexpression paradigm in APP transgenic mice support the hypothesis of a detrimental role for elevated COX2 expression in brains of Alzheimer's disease patients (Xiang *et al.*, 2002). While it has been shown that prostaglandin E₂ levels are increased 5-fold in the CSF of probable Alzheimer's disease patients (Montine *et al.*, 1999), the role of COX products for Alzheimer's disease pathophysiology has not been fully elucidated yet (Akiyama *et al.*, 2000), raising the question of whether the COXs are indeed the biologically relevant target of NSAIDs responsible for the reduced risk of Alzheimer's disease. Moreover, recent clinical trials of COX2 selective inhibitors in Alzheimer's disease patients showed that they are ineffective in treating the disease (Aisen *et al.*, 2003). Therefore, alternative targets of NSAID action have to be considered. A novel action of a subset of commonly used NSAIDs was reported recently by Weggen *et al.* (2001) who found that these drugs selectively reduce the production of A β ₁₋₄₂ due to alteration of the preferred γ -secretase cleavage site in APP.

A subset of NSAIDs directly regulate gene expression through their ability to bind and activate the nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR γ) (Lehmann *et al.*, 1997). In microglia and macrophages, PPAR γ activation results in the inhibition of proinflammatory gene expression through silencing the action of the transcription factors nuclear factor- κ B and AP-1 on the promoters of these genes (Jiang *et al.*, 1998; Ricote *et al.*, 1998; Daynes and Jones, 2002). We have demonstrated that PPAR γ agonists reduce A β - and cytokine-mediated neuroinflammation and neurotoxicity both *in vitro* (Heneka *et al.*, 1999; Combs *et al.*, 2000) and *in vivo* (Heneka *et al.*, 2000). Moreover, we were able to show *in vitro* that NSAIDs, such as indomethacin and ibuprofen, as well as several structurally different PPAR γ agonists, reduce immunostimulated A β production in a PPAR γ -dependent manner (Sastre *et al.*, 2003). We report here that acute treatment of APP-overexpressing mice with ibuprofen or the specific PPAR γ agonist, pioglitazone, results in reduction of microglial activation, inflammatory gene expression, BACE1 levels and A β deposition in the brain.

Material and methods

Animals

The transgenic mice used in this study were of the FVB/N genetic background and expressed APPV717I under the control of the mouse thyl gene promoter. The generation of these APPV717I transgenic mice has been described previously (Moechars *et al.*, 1999). Mice ($n = 6$ per group) of 10 months of age were used for experiments

since APPV717I mice begin to deposit amyloid peptides in the subiculum at this age, with an accompanying profound inflammatory component becoming apparent. Mice were fed Purina 5002 rodent chow *ad libitum* supplemented with either 375 p.p.m. ibuprofen (Sigma, St Louis, MO) or 240 p.p.m. pioglitazone (Actos™ Takeda Pharmaceuticals, Osaka, Japan). The latter drug concentration was chosen since only ~18% of pioglitazone crosses the blood–brain barrier in mammals (Maeshiba *et al.*, 1997), and a previous experiment using a lower drug dosage failed to show statistically significant results (Yan *et al.*, 2003). Animals were treated for 7 days, since this time period was believed to allow the detection of both anti-inflammatory and amyloid-lowering effects. The final dosage of drug was computed to be 62.5 mg/kg/day of ibuprofen and 40 mg/kg/day of pioglitazone based on an average daily food consumption of 5 g of chow per mouse. There were no significant changes in body weight between or within groups of mice. Of note, pioglitazone did not affect blood glucose levels in APP mice as determined in a set of pilot experiments using the identical treatment protocol in accordance with previous observations in non-diabetic mice (Shiomi *et al.*, 2002). During the experimental treatment, animals were housed singly to allow monitoring of drug intake by weighing the animal daily as well as weighing the remaining food pellets at the same time. In all instances, animals lived under standard conditions of 22°C with a 12 h light–dark cycle and with free access to food and water. At the time of sacrifice, animals received a short inhalation anaesthesia using isoflurane. Animals were transcardially perfused with heparinized sodium chloride (0.9%). The brains were removed and brain regions were dissected from one hemisphere. One hemisphere was placed into an acrylic brain block lined up with the interaural line at 0 mm. Subsequently, coronal slices were cut to obtain cortical, hippocampal, cerebellar and brainstem sections, which were dissected, snap-frozen in liquid nitrogen and stored at –80°C until further analysis by reverse transcription–polymerase chain reaction (RT–PCR) within 2 weeks. The remaining brain hemisphere was either fixed in 4% paraformaldehyde followed by paraffin embedding according to standard protocols and sectioning for immunohistochemistry, or underwent cryofixation. For the latter purpose, brains were mounted with Jung tissue freezing medium (Leica Microsystems, Nussloch, Germany), frozen in melting isopentane and stored in liquid nitrogen until further processing. Animal care and handling was performed according to the Declaration of Helsinki and was approved by the local ethics committees (approval #50.203.2BN33,34/00).

Biochemical analysis of transgenic mouse brain

The individual hemispheres were homogenized in 6.5 volumes of ice-cold buffer containing 20 mM Tris–HCl (pH 8.5) and a cocktail of proteinase inhibitors (Roche Mannheim, Germany), using a small Potter-type mechanical homogenizer. After centrifugation (135 000 g for 1 h at 4°C), a portion of the supernatant was centrifuged again (200 000 g for 2 h at 4°C) and soluble amyloid peptides were quantified by enzyme-linked immunosorbent assay (ELISA).

ELISA for soluble and plaque-associated amyloid peptides

The extracted protein fractions were applied to small C18-Sep-Pack reverse phase columns (Waters, Massachusetts, MA) and washed with increasing concentrations of acetonitrile (5, 25 and 50%)

containing 0.1% trifluoroacetic acid (TFA). The last fraction, containing the amyloid peptides, was dried under vacuum overnight. The samples were assayed using a sandwich ELISA for Aβ1–40 and Aβ1–42 peptides. The capture antisera were CFA3340 (Barelli *et al.*, 1997) and 21F12. The reaction was developed with the biotinylated monoclonal antibody 6F3D.

Immunohistochemistry

Serial sagittal sections of paraffin-embedded or cryofixed tissue were cut (7 μm thick, Leica microtome 2155 or Leica Cryostat CM3050S) and mounted (poly-L-lysine-coated slides, Histobond adhesion slides, Marienfeld, Germany). Retrieval of antigen sites, blocking of endogenous peroxidase activity and blocking of non-specific binding sites were performed according to standard protocols. After washing in phosphate-buffered saline (PBS), sections were incubated overnight at 4°C with primary antibodies. The antibodies and dilutions used were: (i) rabbit anti-glial fibrillary acidic protein (GFAP) polyclonal antibody (pAb; Z 334; 1 : 800, DAKO, Hamburg, Germany); (ii) rabbit pAb against inducible nitric oxide synthase (iNOS) (32030; 1 : 150, Transduction Laboratories, Bioscience, Heidelberg, Germany); (iii) anti-COX2 affinity-purified antibody (160126; 1 : 1000; Cayman Chemical, Ann Arbor, MI); (iv) rabbit pAb against Aβ1–42 (#44-344; 1 : 50, Biosource International, Inc., USA); (v) a pAb 7520 directed against the C-terminal domain of BACE1 (a gift from Dr Christian Haass, Adolf-Butenandt-Institute, University of Munich); and (vi) a mouse monoclonal antibody against neuronal nuclei (neuN, #MAB 377; 1 : 500, Chemicon, Hofheim, Germany). Microglial cells were identified by means of biotin-labelled isolectin B4 (IB4), from *Bandeiraea simplicifolia* as described by others (Naujoks-Manteuffel and Niemann, 1994) (1 : 50, Sigma Taufkirchen, Germany). Immunohistochemistry was performed using the avidin–biotin–peroxidase complex method (ABC-Kit, Vector Labs) with 3,3'-diaminobenzidine tetrahydrochloride as chromogen.

All fluorescent double immunostaining was performed on cryofixed sections cut (6 μm) and mounted as described above. Sections were dried at room temperature for 1 h and then fixed in 4% PFA or methanol for 15 min at room temperature. After washing with PBS, the double staining was performed by adding simultaneously both first antibodies, and followed by overnight incubation at 4°C. The goat secondary antibodies (fluorescein DTAF-conjugated anti-rabbit 1 : 150, Texas red-conjugated anti-mouse 1 : 80, Texas red-conjugated anti-rat 1 : 80, Jackson Immuno Research Laboratories, West Grove, PA) were applied sequentially after washing in PBS. Negative controls included non-specific IgG instead of primary antibodies, pre-absorption with the respective cognate peptides (150–200 μg of peptide/ml of antibody working solution), omission of the secondary antibody and absence of immunoreactivity in non-transgenic controls of the respective age.

Confocal laser scanning microscopy

Double-labelled specimens were analysed with a confocal laser scanning microscope (Multiprobe 2001; Molecular Probes, Inc., Eugene, OR) equipped with an Ar/Kr laser with balanced emission at 488, 568 and 647 nm. To achieve an optimal signal-to-noise ratio for each fluorophore, sequential scanning with 568 and 488 nm was used. The digitalized images were then processed with ImageSpace 3.10 software (Molecular Probes, Inc.) on a Silicon Graphics

(Mountain View, CA) power series 310GTX work station. Original section series were subjected to Gaussian filtration to reduce noise and enhance weakly but specifically labelled structures. Original and filtered sections were projected on one plane using a maximum intensity algorithm and in some cases using depth-coding and surface-rendering algorithms.

Quantification of immunohistochemistry

For quantitative image analysis of hippocampal and cortical immunostaining, serial sagittal sections of one hemisphere taken from lateral (+0.5 to +2.25) were examined. IB4, GFAP, iNOS, COX2 and A β 1–42 immunostaining was evaluated on sagittal brain sections of six animals from each group. For each animal, antigens were detected in 10 parallel sections having a defined distance of 70 μ m and showing both the hippocampus and cortex. In each section, the hippocampus and the frontal cortex were evaluated. The total stained area (real area) and integral staining density (sum of all individual optical densities of each pixel in the area being measured) were determined and given as stained area in μ m²/mm² (IB4 and GFAP) or percentage of stained surface per region (A β 1–42). For iNOS and COX2, plaque areas were excluded and the number of stained cells was counted and is shown as immunopositive cells per mm². All images were acquired using a standard light and immunofluorescence microscope (Nikon, Eclipse E-800) connected to a digital camera (Sony, model DXC-9100P, Sony, Köln, Germany) and to a PC system with LUCIA imaging software (LUCIA 32G, version 4.11; Laboratory Imaging, Düsseldorf, Germany). For each animal, average values from all sections were determined. These data were analysed by ANOVA with Tukey's post-tests using SYSTAT software (Systat, Evanston, IL).

RNA preparation and RT-PCR

Total RNA was extracted from brain samples using Trizol reagent according to the manufacturer's procedures (Sigma, St Louis, MO), and RT-PCR was carried out as described (Heneka *et al.*, 2002). The primers used were: iNOS forward 5'-TGG GAG CCA CAG CAA TAT AG-3' and iNOS reverse 5'-ACA GTT TGG TGT GGT GTA GG-3'; GFAP forward 5'-TCC GCG GCA CGA ACG AGT C-3' and GFAP reverse 5'-CAC CAT CCC GCA TCT CCA CAG TCT-3'; COX2 forward 5'-AAC ATC CCC TTC CTG CGA AG-3' and COX2 reverse 5'-AAG TCC ACT CCA TGG CCC AG-3'; BACE1 forward 5'-GGCGGGAGTGGTATTATGAGGTGA-3' and BACE1 reverse 5'-TATTGCTGCGGAAGGATGGTGA-3'; GAPDH forward 5'-TCA CCA GGG CTG CCA TTT GC-3' and GAPDH reverse 5'-GAC TCC ACG ACA TAC TCA GC-3'. PCRs were carried out on RNA prepared from the individual animals in each group, and representative gels of two different animals per group are shown. PCR conditions were 35 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 45 s, and extension at 72°C for 45 s using a PX2 thermocycler (ThermoHybaid, Ulm, Germany). PCR products were separated by electrophoresis through 2% agarose containing 0.5 μ g/ml ethidium bromide, imaged using an Alpha Inotech imaging system (Temecula, CA), and band intensities determined using ImageJ public domain software from NIH. The data were analysed by ANOVA with Tukey's post-tests (Systat, Evanston, IL).

Results

Anti-inflammatory drug treatment reduces glial activation

The 10-month-old APPV717I transgenic mice received an oral treatment with either standard chow, or chow containing the PPAR γ agonist pioglitazone or with ibuprofen for a period of 7 days. At 10 months of age, the APPV717I mice are starting to exhibit focal deposits of amyloid, accompanied by a glia-mediated inflammatory response. Analysis of sections of the hippocampus and frontal cortex revealed that treatment with ibuprofen or pioglitazone reduced the area covered by IB4-positive microglia by 30 and 40%, respectively. Additionally, both drugs reduced the integral staining intensity of immunopositive cells (Fig. 1A). Confocal immunolabelling showed that clusters of IB4-stained microglia were closely surrounded by GFAP-positive astrocytes (Fig. 1B). Within these clusters, arborized as well as rounded IB4-positive microglia, indicating different stages of inflammatory activation, were found. A subset of IB4-positive microglial cells also expressed COX2 as determined by confocal laser microscopy (Fig. 1C). The vast majority of COX2-positive microglial cells showed a round to oval morphology, indicating an inflammatory activated state. Quantitative counting of activated and COX2-positive microglial cells in the hippocampus and frontal cortex of placebo- and drug-treated APPV717I mice showed that both ibuprofen and pioglitazone treatment effectively reduced the number of COX2-positive microglia in both brain regions. In parallel, COX2 mRNA levels in hippocampal lysates were significantly decreased by ibuprofen and pioglitazone treatment (Fig. 1C).

To determine whether astrocyte activation is influenced by drug treatment, immunohistochemical analysis of GFAP, an astrocyte-specific intermediate filament component, was performed. GFAP expression previously has been reported to be upregulated in APP-overexpressing mice by ~50% (Lim *et al.*, 2000) and is associated with astrocytosis accompanying amyloid deposition in these animals (Bondolfi *et al.*, 2002). Evaluation of sections of the hippocampus and frontal cortex showed that the area covered by GFAP-positive astrocytes and the respective integral staining intensities were reduced by ibuprofen and pioglitazone treatment (Fig. 2A and B). Co-staining of GFAP and A β 1–42 confirmed that astrocyte activation could be observed predominantly at sites of amyloid deposition (Fig. 2C). Confocal immunostaining for iNOS and GFAP showed that a subset of astrocytes expressed iNOS. These iNOS-positive cells were found in close contact with amyloid plaques (not shown). In addition, an increased immunostaining against nitrotyrosine, indicating NO-dependent peroxynitrite generation, was found near to amyloid deposits (not shown). Detection of GFAP mRNA levels showed that both ibuprofen and pioglitazone reduced GFAP mRNA by ~60%. Pioglitazone-treated animals exhibited a statistically significant 40% decrease in iNOS mRNA, whereas ibuprofen treatment did not reduce iNOS mRNA

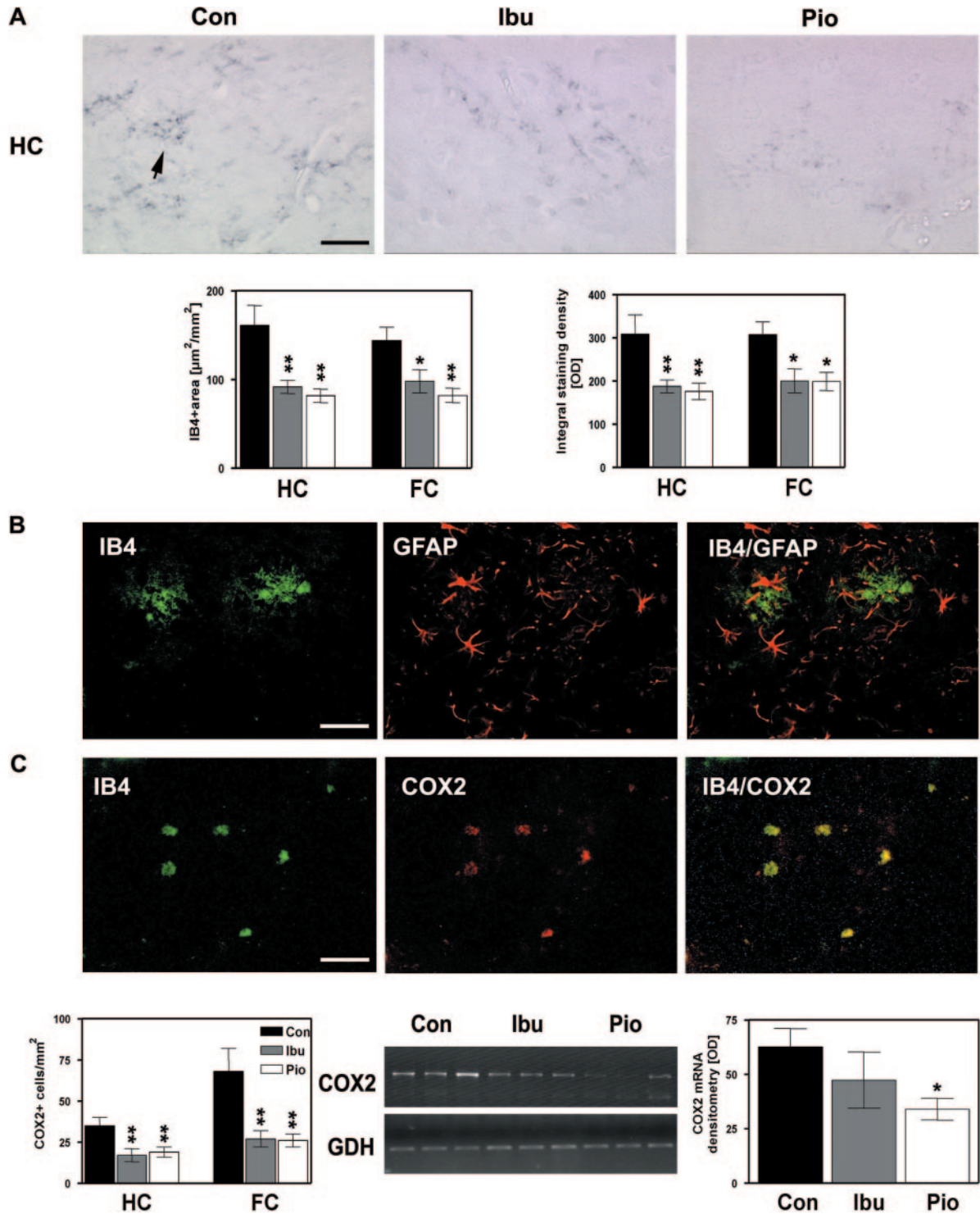


Fig. 1 Anti-inflammatory drug treatment reduces the number of reactive microglia in the hippocampus of APPV717I transgenic mice (10 months of age) received either control chow (Con), or chow supplemented with ibuprofen (Ibu) or pioglitazone (Pio) for 7 days. Activated microglia were visualized by staining with IB4 and are shown in the hippocampus (A). Arrows indicate clusters of microglia cells. For quantitation, the total area covered by IB4 and the integral IB4 staining intensity were determined in serial sections of the hippocampus (HC) and frontal cortex (FC) (A). Confocal analysis of IB4 and the astrocytic marker GFAP revealed that clusters of microglia were closely surrounded by GFAP-positive astrocytes (B). Further co-staining showed that a subset of IB4-positive microglial cells were also expressing cyclooxygenase 2 (COX2), and the number of COX2-positive cells was quantified in the HC and FC (C). COX2 mRNA levels were determined by RT-PCR in hippocampal lysates of all animal groups (displayed as triplicates) and subsequently analysed by densitometry. GAPDH (GDH) served as control (C). Asterisks indicate significant differences between control and drug-treated groups (* $P < 0.05$, ** $P < 0.01$, SEM, ANOVA followed by a Tukey test). (A) Bar = 25 μm , (B) bar = 50 μm , (C) bar = 25 μm .

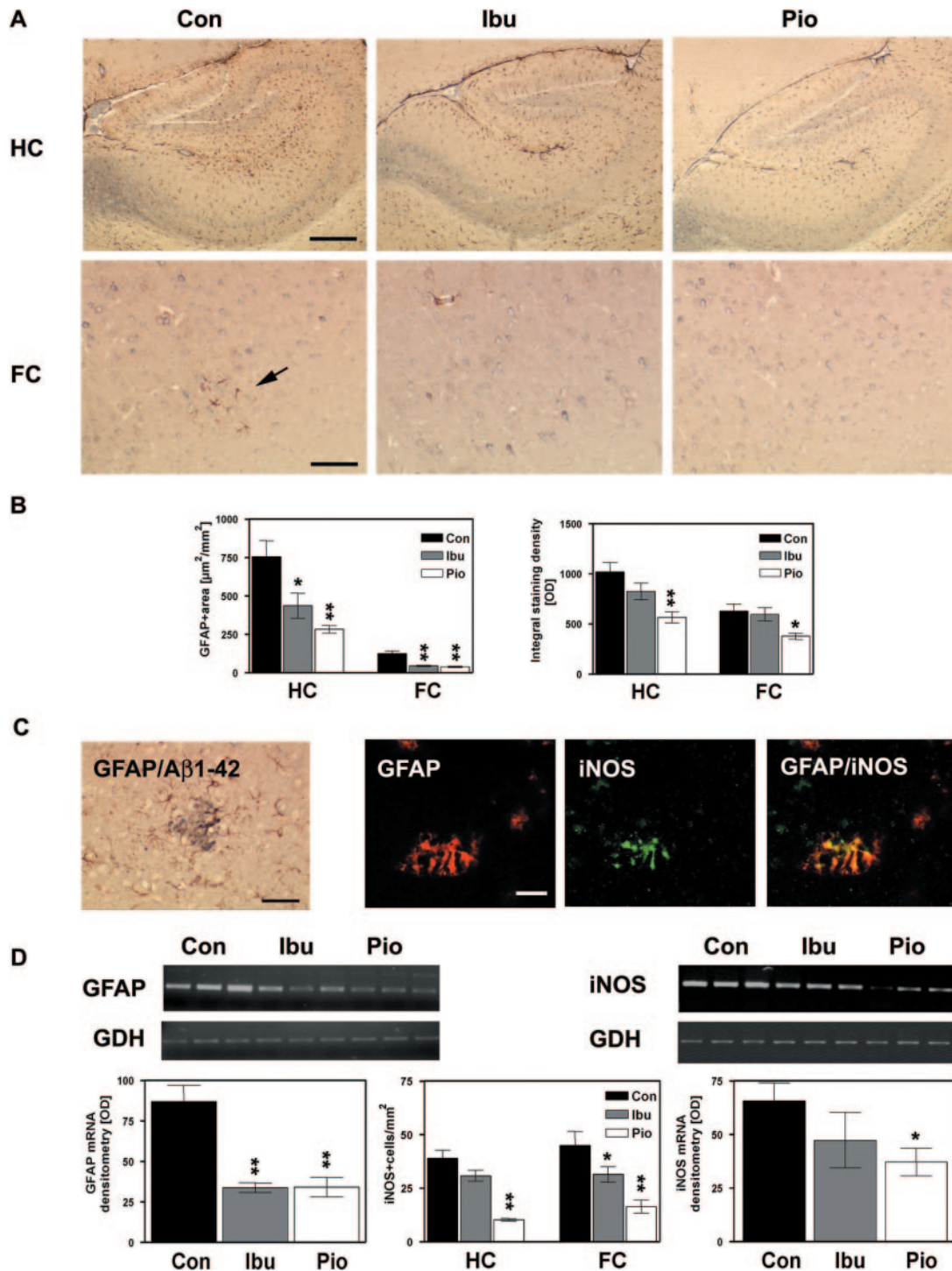


Fig. 2 Downregulation of GFAP expression in 10-month-old APPV717I transgenic mice by anti-inflammatory drug treatment. GFAP expression was analysed immunohistochemically in the hippocampus (HC) and frontal cortex (FC) of 10-month-old APPV717I transgenic mice treated with either control chow (Con), or chow supplemented with ibuprofen (Ibu) or pioglitazone (Pio) for 7 days (A). Arrows indicate clusters of GFAP-positive astrocytes in the FC. For quantitation, the total area covered by GFAP and the integral GFAP staining intensity were determined for both areas, the HC and FC, in serial sections (B). Representative picture of a section co-stained for GFAP (brown) and A β 1-42 (blue), revealing that astrocytes assembled predominantly at sites of amyloid plaque deposition (C). Confocal staining of GFAP and inducible nitric oxide synthase (iNOS) showed that a subset of GFAP-positive cells expressed iNOS (C) and the number of iNOS positive astrocytes was quantified (D). iNOS and GFAP mRNA levels were then determined by RT-PCR in hippocampal lysates of all animal groups (displayed as triplicates) and subsequently analysed by densitometry. GAPDH (GDH) served as control (D). Asterisks indicate significant differences between placebo- and drug-treated groups (* $P < 0.05$, ** $P < 0.01$, SEM, ANOVA followed by Tukey test). (A) Bar = 250 μm (HC) and 100 μm (FC), (C) bar = 50 μm (GFAP/A β 1-42) and 25 μm (GFAP/iNOS).

levels to a comparable extent but tended to be lower than in control animals. The variation observed in the degree of GFAP and iNOS mRNA inhibition upon ibuprofen or pioglitazone treatment between single animals may be due to inter-animal variability in this particular assay but may also result from different drug concentrations at the time of sacrifice. Because chronic amyloid deposition will result in a constant inflammatory stimulus, the degree of inhibition may depend on the individual food intake, especially in food-based treatment protocols. However, immunohistochemical quantification of iNOS-positive cells found a corresponding reduction of the number of iNOS-positive astrocytes in response to drug treatment (Fig. 2D).

Reduced levels of BACE1 protein and mRNA in pioglitazone-treated APPV717I mice

We previously have reported that treatment of neuroblastoma cells with several proinflammatory cytokines resulted in an increased BACE1 expression and that ibuprofen and pioglitazone reduced immunostimulated BACE1 levels *in vitro* (Sastre *et al.*, 2003). These data prompted us to perform an immunohistochemical analysis of BACE1 expression and detection of BACE1 mRNA levels. BACE1-positive cells were immunohistochemically detected in the frontal cortex (Fig. 3A) and hippocampus of 10-month-old APPV717I

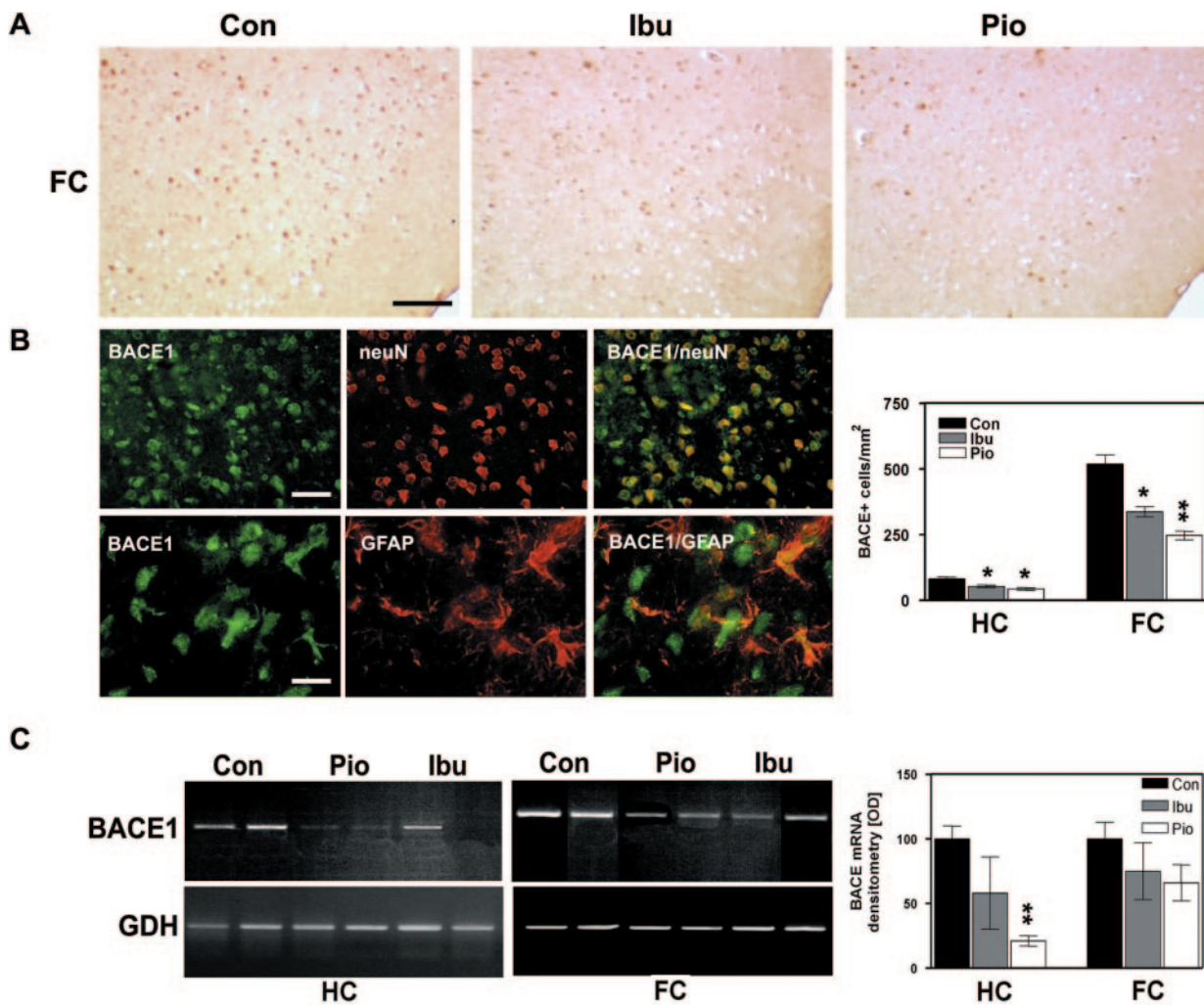


Fig. 3 Pioglitazone decreases the level of BACE1 mRNA and protein in APPV717I-transgenic mice. Immunohistochemical detection of β -secretase (BACE1) in the frontal cortex (FC) of 10-month-old APPV717I transgenic mice treated with either control chow (Con), or chow supplemented with ibuprofen (Ibu) or pioglitazone (Pio) for 7 days (A). Confocal immunostaining of BACE1 and the neuronal marker neuN revealed that BACE1 was mostly expressed by neurons in 10-month-old APPV717I mice (B). However, co-staining of BACE1 and GFAP showed that a subset of astrocytes also expressed BACE1 (B). Quantification of BACE1-positive cells showed a significant reduction in response to both pioglitazone and ibuprofen treatment (B). BACE1 mRNA levels were determined in lysates of the hippocampus (HC) and subsequently analysed by densitometry (C). Asterisks indicate significant differences between the control and drug-treated groups ($P < 0.05$, $**P < 0.01$, SEM, ANOVA followed by Tukey test). (A) Bar = 250 μ m, (B) bar = 50 μ m (BACE1/neuN) and 25 μ m (BACE1/GFAP).

transgenic mice. At this age, APPV717I mice expressed BACE1 mainly in neurons as determined by confocal immunohistochemistry for the neuronal marker neuN and BACE1 (Fig. 3B). In addition, co-staining of sections with GFAP revealed that activated astrocytes were found in focal clusters, with a subset of astrocytes also expressing BACE1 (Fig. 3B). Quantification of BACE1-positive neurons in ibuprofen- or pioglitazone-treated animals revealed a marked reduction in the frontal cortex and hippocampus when compared with non-treated APP mice (Fig. 3B). Analysis of BACE1 mRNA levels in whole brain lysates from the respective brain regions showed that pioglitazone, and to a lesser extent ibuprofen, downregulated BACE1 mRNA (Fig. 3C).

Pioglitazone and ibuprofen drug treatment alters A β levels and deposition

Anti-inflammatory drug treatment has been reported to reduce A β levels in the brain of APP-expressing mice (Lim *et al.*, 2000, 2001; Weggen *et al.*, 2001; Yan *et al.*, 2003). The APPV717I-transgenic mice investigated in the present study start to exhibit A β deposition at 10 months of age (Moechars *et al.*, 1999; Van Dorpe *et al.*, 2000). Immunohistochemical analysis of A β deposits in the hippocampus and frontal cortex was performed using an antibody specific for A β 1–42 (Fig. 4A). Compared with non-treated APP mice, pioglitazone reduced the percentage of A β 1–42-stained plaque area by ~25 and 33% in the hippocampus and frontal cortex, respectively (Fig. 4B). Ibuprofen exerted a similar reduction in the hippocampus; however, it was less efficient in reducing amyloid deposition in the frontal cortex. In addition, there was a corresponding significant reduction of A β 1–42 staining intensity in the pioglitazone-treated animals (Fig. 4B). We measured soluble levels of A β 1–40 and A β 1–42 by ELISA and found that treatment with pioglitazone significantly reduced cerebral levels of soluble A β 1–42 by 27% (Fig. 4C). There was a smaller and statistically insignificant reduction in ibuprofen-treated mice. Soluble A β 1–40 levels were not significantly altered by drug treatment over this interval (Fig. 4C). The A β 42/40 ratio was reduced 22% in pioglitazone-treated animals (Fig. 4C).

Discussion

There recently has been an increased appreciation of the role that inflammation plays in the pathogenesis of Alzheimer's disease that has arisen principally from epidemiological studies showing a dramatic effect of long-term NSAID treatment on Alzheimer's disease risk. However, the molecular mechanisms by which NSAIDs intervene in the pathological processes that underlie cognitive decline and neuronal loss remain unclear (Stewart *et al.*, 1997; In 't Veld *et al.*, 2001).

We show that a brief and oral treatment of 10-month-old APPV717I-overexpressing mice with the PPAR γ agonist pioglitazone and with the NSAID ibuprofen decreased

amyloid-associated microglial and astroglial activation in the hippocampus and frontal cortex. In particular, microglial COX2 expression as well as astrocytic expression of iNOS were potentially reduced by both treatments. BACE1, a key enzyme of the APP processing pathway, was detected predominantly in neurons and to a minor extent in clustered astrocytes, and found to be significantly reduced by both pioglitazone and ibuprofen treatment. In parallel to BACE1 reduction, pioglitazone, and to a lesser extent ibuprofen, were able to decrease the total A β 1–42-positive amyloid plaque area and the respective staining intensity. Additionally, soluble levels of A β 1–42 were significantly attenuated by pioglitazone treatment.

Animal models of Alzheimer's disease which develop extensive amyloid deposits in the brain also exhibit reactive astrocytosis and abundant plaque-associated, activated microglia with elevated levels of proinflammatory secretory products (Frautschy *et al.*, 1998; Benzing *et al.*, 1999; Stalder *et al.*, 1999; Mehlhorn *et al.*, 2000; Bornemann *et al.*, 2001; Matsuoka *et al.*, 2001). Two recent studies in Tg2576 animals have demonstrated that ibuprofen treatment over a period of several months dramatically reduces glial activation and proinflammatory gene expression (Lim *et al.*, 2000; Yan *et al.*, 2003). These studies suggest that anti-inflammatory treatment strategies may offer substantial clinical benefit. However, several recent clinical trials using COX2-specific inhibitors (Aisen, 2002; Aisen *et al.*, 2003) and traditional NSAIDs alone or in combination (Aisen, 2002) have failed, suggesting that we do not yet fully understand the biology underlying the inflammatory processes in Alzheimer's disease or the relevant mechanisms of protective drug actions, and suggesting that alternative mechanisms of NSAID drug action have to be considered.

It has been recognized recently that a subset of NSAIDs can bind to and activate the nuclear hormone receptor PPAR γ (Lehmann *et al.*, 1997; Jaradat *et al.*, 2001). In myeloid lineage cells, including microglia, the principal effect of PPAR γ activation is to transcriptionally silence proinflammatory gene expression (Jiang *et al.*, 1998; Ricote *et al.*, 1998; Daynes and Jones, 2002). We have argued that the anti-inflammatory effects of NSAIDs may be partly mediated through the activation of this transcription factor (Landreth and Heneka, 2001). Anti-inflammatory effects of PPAR γ agonists and ibuprofen have been shown to protect neurons from cytokine-mediated death (Heneka *et al.*, 1999) and were also observed following infusion of immunostimulants into rodent brain (Heneka *et al.*, 2000). Studying A β -mediated microglial activation and neurotoxicity *in vitro*, Combs *et al.* reported that PPAR γ agonists, including ibuprofen, were neuroprotective (Combs *et al.*, 2000). These findings led us to investigate the effects of a highly specific PPAR γ agonist and ibuprofen in APPV717I-overexpressing mice, a murine model of the amyloid pathology in Alzheimer's disease (Van Leuven, 2000). We found that acute treatment of these animals with the PPAR γ agonist pioglitazone resulted in a significant reduction in the number of IB4-positive microglia

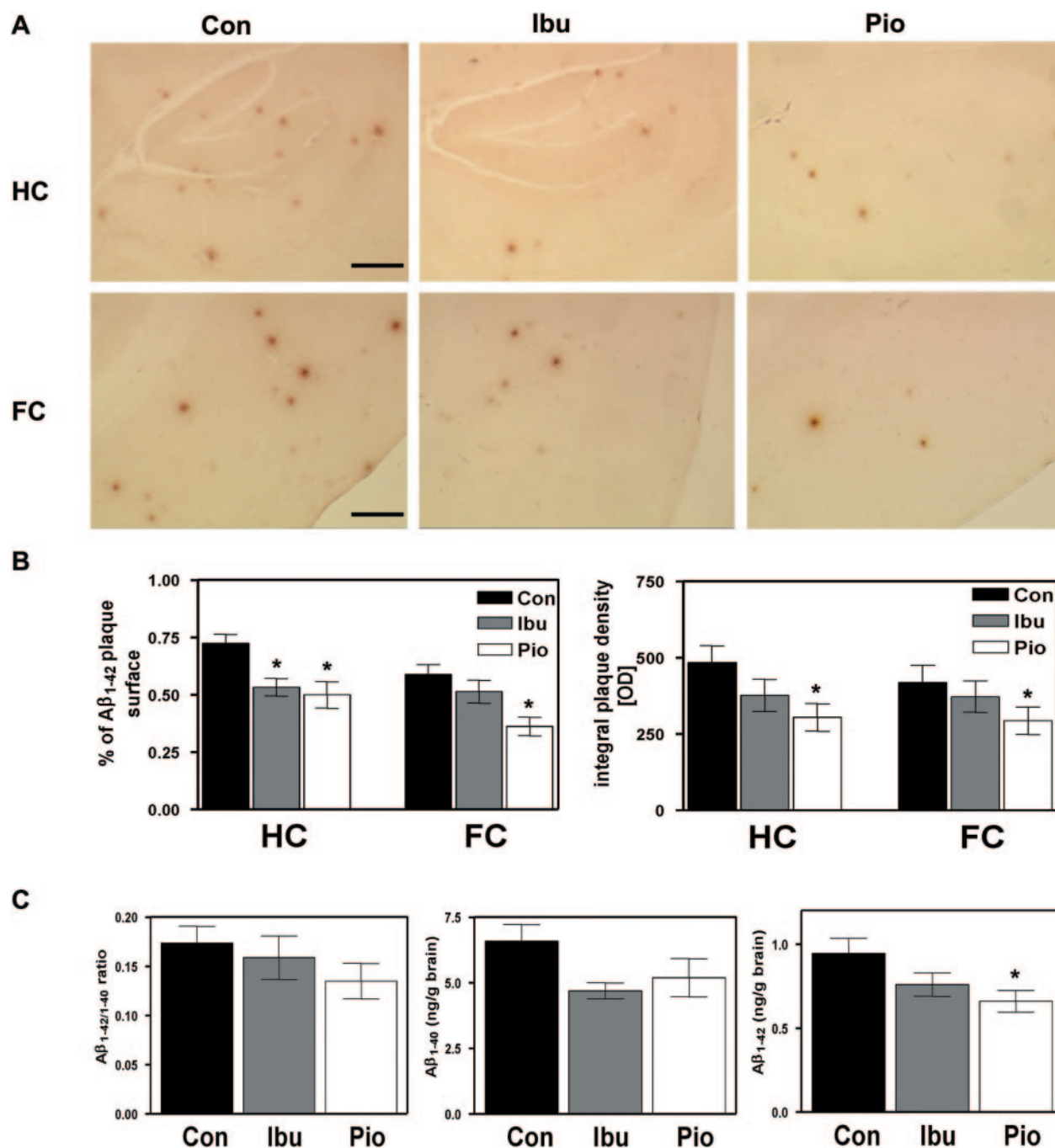


Fig. 4 Effect of anti-inflammatory drug treatment on Aβ levels in 10-month-old APPV717I transgenic mice. Immunohistochemical analysis of Aβ₁₋₄₂ deposition in the hippocampus (HC) and frontal cortex (FC) was performed in 10-month-old APPV717I transgenic mice treated with either control chow (Con), or chow supplemented with ibuprofen (Ibu) or pioglitazone (Pio) for 7 days (A). Serial sections were evaluated for the total plaque surface (given as a percentage of Aβ₁₋₄₂-stained surface/region) and for the integral Aβ₁₋₄₂ staining density in the HC and FC (B). Levels of soluble Aβ₁₋₄₂, Aβ₁₋₄₀ and the ratio Aβ₁₋₄₂/Aβ₁₋₄₀ were quantitated by ELISA (C). Aβ levels are given as ng/g brain tissue. Asterisks indicate significant differences between the control and drug-treated groups (**P* < 0.05, SEM, ANOVA followed by Tukey test). (A) Bar = 250 μm.

and GFAP-positive astrocytes in the hippocampus and the frontal cortex. Moreover, the brief anti-inflammatory drug treatment inhibited the transcription and expression of COX2 and iNOS in both brain areas. COX2 was expressed predominantly in activated microglial cells, whereas iNOS

was strictly co-localized with GFAP, indicating that activated astrocytes served as a major source of iNOS-dependent NO. Inhibition of both COX2 and iNOS has been proven beneficial in several other models of neurodegeneration and inflammation including amyotrophic lateral sclerosis, brain trauma

and experimental stroke (Zhang *et al.*, 1996; Iadecola *et al.*, 2001; Parmentier-Batteur *et al.*, 2001; Hurley *et al.*, 2002; Pompl *et al.*, 2003; Manabe *et al.*, 2004), suggesting that pioglitazone, and to a lesser extent ibuprofen, can exert neuroprotective effects. Similar results showing that ibuprofen treatment reduced activated, plaque-associated microglia as well as interleukin (IL)-1 β levels were obtained after an oral treatment of APP-overexpressing mice for 6 months (Lim *et al.*, 2000). One of the most important outcomes of the present study is that short intervals of drug treatment inhibit the expression of inflammatory markers and reduce the activation status of microglia and astrocytes. In this respect, it seems noteworthy that similarly to the findings in previous studies and the present study, evaluation of human pathological material indicates that NSAID treatment results in a reduction of glial activation that accompanies amyloid deposition and plaque formation in the brains of Alzheimer's disease patients (Mackenzie and Munoz, 1998; Alafuzoff *et al.*, 2000). While pioglitazone and ibuprofen share the pharmacological mechanism of PPAR γ activation, it is not clear whether both drugs exert their beneficial effects through activation of this receptor. Pioglitazone and other thiazolidinediones have also been suggested to exert their anti-inflammatory effects through PPAR γ -independent mechanisms (Chawla *et al.*, 2001) and ibuprofen has been shown to inhibit protein kinases that are elements of inflammatory signalling cascades and nuclear factor- κ B (Tegeger *et al.*, 2001).

Inflammation plays a role in amyloidogenesis and A β deposition; however, the mechanistic relationship between these processes remains to be resolved. The capacity of inflammatory stimuli to promote the generation of amyloidogenic A β species has not been examined extensively; however, Blasko and colleagues have reported that neuronal A β secretion is enhanced by cytokine exposure (Blasko *et al.*, 2001). These findings were recently extended by showing that several cytokines including IL-1 β and tumour necrosis factor- α , alone or in concert, effectively increase the generation of the fibrillogenic A β 1–40 and A β 1–42 peptides *in vitro* (Sastre *et al.*, 2003). The observed increase of amyloid secretion was paralleled *in vitro* by an upregulation of BACE1, one of two key secretases of the APP processing pathway. In the present study, BACE1 was mainly expressed by neurons but was also focally found in astrocytes as revealed by confocal immunostaining. Treatment of animals with pioglitazone and ibuprofen reduced the number of BACE1-positive neurons and astrocytes and BACE1 mRNA levels in both brain regions, the hippocampus and frontal cortex.

In the present study, a 7 day treatment with pioglitazone and ibuprofen decreased the area of focal A β 1–42-positive amyloid deposits and their respective staining intensity in the hippocampus and frontal cortex. This latter finding confirms previous reports in ibuprofen- and indomethacin-treated animals. However, in all previous studies, treatment intervals were considerably longer (Lim *et al.*, 2000; Jantzen *et al.*,

2002; Quinn *et al.*, 2003; Yan *et al.*, 2003). While PPAR γ -independent mechanisms need to be considered, *in vitro* data showing that ibuprofen and pioglitazone as well as other NSAIDs and structurally different PPAR γ agonists share a common and PPAR γ -dependent mechanism in reducing immunostimulated BACE1 and A β production (Sastre *et al.*, 2003) suggest that at least one dimension of NSAID-mediated protection is mediated by PPAR γ .

An unexpected finding was the observation that acute treatment with pioglitazone resulted in a significant 27% reduction of brain soluble A β 1–42 levels. The only comparable data on acute effects of anti-inflammatory drugs on A β levels in transgenic mice are those of Weggen *et al.* (Weggen *et al.*, 2001; Eriksen *et al.*, 2003). These authors reported that oral treatment of 3-month-old Tg2576 mice with ibuprofen (50 mg/kg/day) for only 3 days resulted in a reduction of SDS-soluble A β 1–42 levels by \sim 40% without a change in A β 1–40. In the present study, we observed a reduction in soluble A β levels with 7 days of ibuprofen treatment; however, this effect did not reach statistical significance. Weggen and colleagues have shown that ibuprofen and other NSAIDs can alter APP processing, and this effect is correlated with the ability of ibuprofen to reduce A β 1–42 levels acutely in APP-expressing transgenic mice (Weggen *et al.*, 2001; Sagi *et al.*, 2003; Yan *et al.*, 2003). These findings support the view that these drugs act on γ -secretase to alter its specificity. The effects of pioglitazone on A β deposition are not likely to arise from a direct effect on γ -secretase, as postulated for ibuprofen, as we and others have been unable to demonstrate an effect of pioglitazone or other PPAR γ agonists on γ -secretase-dependent processing using *in vitro* assay systems (Sastre *et al.*, 2003). Importantly, the drug concentrations used to affect γ -secretase *in vitro* are far higher than those observed in human CSF samples after oral or intramuscular NSAID application (Bannwarth *et al.* 1989, 1995; Weggen *et al.*, 2001). In contrast, the observed CSF levels of NSAIDs are exactly in the range (low micromolar concentrations) where they start to act as effective PPAR γ activators (Jaradat *et al.*, 2001; Kojo *et al.*, 2003).

In a previous study using APP transgenic mice, pioglitazone had failed to reduce amyloid levels significantly, even when administered for a longer period (Yan *et al.*, 2003). However, since only \sim 18% of orally administered pioglitazone crosses the intact blood–brain barrier in mammals (Maeshiba *et al.*, 1997), it seems likely that drug dosage, rather than treatment duration, is critical to observe drug effects in the brain. While Yan and colleagues observed a strong tendency of pioglitazone to reduce A β 1–42 levels, we found a significant and strong reduction of soluble and deposited A β 1–42 levels using twice the drug concentrations employed in the previous study.

The principal clinical usage of PPAR γ agonists is for treatment of type II diabetes. The drugs act to enhance insulin sensitivity and normalize blood glucose levels (Patsouris *et al.*, 2004; Yki-Jarvinen, 2004). It is possible that the reported salutary effects of PPAR γ might arise from improved

brain glucose utilization in addition to the anti-inflammatory actions of these drugs (Watson and Craft, 2003). Since insulin resistance increases amyloidosis in APP transgenic mice (Ho *et al.*, 2004), the amyloid-lowering effect of pioglitazone observed in this study may also be due to enhanced insulin sensitivity.

The data reported here support the use of PPAR γ agonists in the treatment of Alzheimer's disease. Recent studies have documented the salutary effects of PPAR γ agonists in animal models of multiple sclerosis (Niino *et al.*, 2001; Diab *et al.*, 2002; Feinstein *et al.*, 2002; Natarajan and Bright, 2002) and Parkinson's disease (Braidert *et al.*, 2002). The potent anti-inflammatory effects of PPAR γ agonists suggest that they may have beneficial effects in treating other CNS diseases with an inflammatory component.

Acknowledgements

This study was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 4000, A8) to M.T.H. and T.K., and the NIH (AG 16740, AG008012), the Blanchett Hooker Rockefeller Foundation and the CART Fund of Rotary International to G.L.

References

- Aisen PS. Evaluation of selective COX-2 inhibitors for the treatment of Alzheimer's disease. *J Pain Symptom Manag* 2002; 23: S35–40.
- Aisen PS, Schafer KA, Grundman M, et al. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. *J Am Med Assoc* 2003; 289: 2819–26.
- Akiyama H, Barger S, Barnum S, et al. Inflammation and Alzheimer's disease. *Neurobiol Aging* 2000; 21: 383–421.
- Alafuzoff I, Overmyer M, Helisalmi S, Soyninen H. Lower counts of astroglia and activated microglia in patients with Alzheimer's disease with regular use of non-steroidal anti-inflammatory drugs. *J Alzheimers Dis* 2000; 2: 37–46.
- Bannwarth B, Lopicque F, Pehourcq F, et al. Stereoselective disposition of ibuprofen enantiomers in human cerebrospinal fluid. *Br J Clin Pharmacol* 1995; 40: 266–9.
- Bannwarth B, Netter P, Poullet J, Royer RJ, Gaucher A. Clinical pharmacokinetics of nonsteroidal anti-inflammatory drugs in the cerebrospinal fluid. *Biomed Pharmacother* 1989; 43: 121–6.
- Barelli H, Lebeau A, Vizzavona J, et al. Characterization of new polyclonal antibodies specific for 40 and 42 amino acid-long amyloid beta peptides: their use to examine the cell biology of presenilins and the immunohistochemistry of sporadic Alzheimer's disease and cerebral amyloid angiopathy cases. *Mol Med* 1997; 3: 695–707.
- Benzing WC, Wujek JR, Ward EK, et al. Evidence for glial-mediated inflammation in aged APP(SW) transgenic mice. *Neurobiol Aging* 1999; 20: 581–9.
- Blasko I, Apochal A, Boeck G, Hartmann T, Grubeck-Loebenstien B, Ransmayr G. Ibuprofen decreases cytokine-induced amyloid beta production in neuronal cells. *Neurobiol Dis* 2001; 8: 1094–101.
- Bondolfi L, Calhoun M, Ermini F, et al. Amyloid-associated neuron loss and gliogenesis in the neocortex of amyloid precursor protein transgenic mice. *J Neurosci* 2002; 22: 515–22.
- Bornemann KD, Wiederhold KH, Pauli C, et al. Abeta-induced inflammatory processes in microglia cells of APP23 transgenic mice. *Am J Pathol* 2001; 158: 63–73.
- Braidert T, Callebert J, Heneka MT, Landreth G, Launay JM, Hirsch EC. Protective action of the peroxisome proliferator-activated receptor-gamma agonist pioglitazone in a mouse model of Parkinson's disease. *J Neurochem* 2002; 82: 615–24.
- Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat Med* 2001; 7: 48–52.
- Combs CK, Johnson DE, Karlo JC, Cannady SB, Landreth GE. Inflammatory mechanisms in Alzheimer's disease: inhibition of beta-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists. *J Neurosci* 2000; 20: 558–67.
- Daynes RA, Jones DC. Emerging roles of PPARs in inflammation and immunity. *Nat Rev Immunol* 2002; 2: 748–59.
- Diab A, Deng C, Smith JD, et al. Peroxisome proliferator-activated receptor-gamma agonist 15-deoxy-Delta(12,14)-prostaglandin J(2) ameliorates experimental autoimmune encephalomyelitis. *J Immunol* 2002; 168: 2508–15.
- Dingwall C. Spotlight on BACE: the secretases as targets for treatment in Alzheimer disease. *J Clin Invest* 2001; 108: 1243–6.
- Eriksen JL, Sagi SA, Smith TE, et al. NSAIDs and enantiomers of flurbiprofen target gamma-secretase and lower Abeta 42 *in vivo*. *J Clin Invest* 2003; 112: 440–9.
- Esler WP, Wolfe MS. A portrait of Alzheimer secretases—new features and familiar faces. *Science* 2001; 293: 1449–54.
- Feinstein DL, Galea E, Gavriluyk V, et al. Peroxisome proliferator-activated receptor-gamma agonists prevent experimental autoimmune encephalomyelitis. *Ann Neurol* 2002; 51: 694–702.
- Frautschy SA, Yang F, Irrizarry M, et al. Microglial response to amyloid plaques in APPsw transgenic mice. *Am J Pathol* 1998; 152: 307–17.
- Griffin WS, Sheng JG, Royston MC, et al. Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. *Brain Pathol* 1998; 8: 65–72.
- Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol Sci* 1991; 12: 383–8.
- Heneka MT, Feinstein DL, Galea E, Gleichmann M, Wullner U, Klockgether T. Peroxisome proliferator-activated receptor gamma agonists protect cerebellar granule cells from cytokine-induced apoptotic cell death by inhibition of inducible nitric oxide synthase. *J Neuroimmunol* 1999; 100: 156–68.
- Heneka MT, Klockgether T, Feinstein DL. Peroxisome proliferator-activated receptor-gamma ligands reduce neuronal inducible nitric oxide synthase expression and cell death *in vivo*. *J Neurosci* 2000; 20: 6862–7.
- Heneka MT, Galea E, Gavriluyk V, et al. Noradrenergic depletion potentiates beta-amyloid-induced cortical inflammation: implications for Alzheimer's disease. *J Neurosci* 2002; 22: 2434–42.
- Ho L, Qin W, Pompl PN, et al. Diet-induced insulin resistance promotes amyloidosis in a transgenic mouse model of Alzheimer's disease. *FASEB J* 2004; 18: 902–4.
- Hsiao KK, Borchelt DR, Olson K, et al. Age-related CNS disorder and early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. *Neuron* 1995; 15: 1203–18.
- Hurley SD, Olschowka JA, O'Banion MK. Cyclooxygenase inhibition as a strategy to ameliorate brain injury. *J Neurotrauma* 2002; 19: 1–15.
- Iadecola C, Niwa K, Nogawa S, et al. Reduced susceptibility to ischemic brain injury and N-methyl-D-aspartate-mediated neurotoxicity in cyclooxygenase-2-deficient mice. *Proc Natl Acad Sci USA* 2001; 98: 1294–9.
- In 't Veld, Ruitenbergh A, Hofman A, et al. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. *N Engl J Med* 2001; 345: 1515–21.
- Jantzen PT, Connor KE, DiCarlo G, et al. Microglial activation and beta-amyloid deposit reduction caused by a nitric oxide-releasing nonsteroidal anti-inflammatory drug in amyloid precursor protein plus presenilin-1 transgenic mice. *J Neurosci* 2002; 22: 2246–54.
- Jaradat MS, Wongsud B, Phornchirasilp S, et al. Activation of peroxisome proliferator-activated receptor isoforms and inhibition of prostaglandin H(2) synthases by ibuprofen, naproxen, and indomethacin. *Biochem Pharmacol* 2001; 62: 1587–95.
- Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998; 391: 82–6.

- Kojo H, Fukagawa M, Tajima K, et al. Evaluation of human peroxisome proliferator-activated receptor (PPAR) subtype selectivity of a variety of anti-inflammatory drugs based on a novel assay for PPAR delta(beta). *J Pharmacol Sci* 2003; 93: 347–55.
- Lamb BT, Bardel KA, Kulnane LS, et al. Amyloid production and deposition in mutant amyloid precursor protein and presenilin-1 yeast artificial chromosome transgenic mice. *Nat Neurosci* 1999; 2: 695–7.
- Landreth GE, Heneka MT. Anti-inflammatory actions of peroxisome proliferator-activated receptor gamma agonists in Alzheimer's disease. *Neurobiol Aging* 2001; 22: 937–44.
- Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. *J Biol Chem* 1997; 272: 3406–10.
- Lim GP, Yang F, Chu T, et al. Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. *J Neurosci* 2000; 20: 5709–14.
- Lim GP, Yang F, Chu T, et al. Ibuprofen effects on Alzheimer pathology and open field activity in APPsw transgenic mice. *Neurobiol Aging* 2001; 22: 983–91.
- Mackenzie IR, Munoz DG. Nonsteroidal anti-inflammatory drug use and Alzheimer-type pathology in aging. *Neurology* 1998; 50: 986–90.
- Maeshiba Y, Kiyota Y, Yamashita K, Yoshimura Y, Motohashi M, Tanayama S. Disposition of the new antidiabetic agent pioglitazone in rats, dogs, and monkeys. *Arzneimittel-Forschung* 1997; 47: 29–35.
- Manabe Y, Anrather J, Kawano T, et al. Prostanoids, not reactive oxygen species, mediate COX-2-dependent neurotoxicity. *Ann Neurol* 2004; 55: 668–75.
- Matsuoka Y, Picciano M, Malester B, et al. Inflammatory responses to amyloidosis in a transgenic mouse model of Alzheimer's disease. *Am J Pathol* 2001; 158: 1345–54.
- McGeer PL, Schulzer M, McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology* 1996; 47: 425–32.
- Mehlhorn G, Hollborn M, Schliebs R. Induction of cytokines in glial cells surrounding cortical beta-amyloid plaques in transgenic Tg2576 mice with Alzheimer pathology. *Int J Dev Neurosci* 2000; 18: 423–31.
- Moechars D, Dewachter I, Lorent K, et al. Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. *J Biol Chem* 1999; 274: 6483–92.
- Montine TJ, Sidell KR, Crews BC, et al. Elevated CSF prostaglandin E2 levels in patients with probable AD. *Neurology* 1999; 53: 1495–8.
- Natarajan C, Bright JJ. Peroxisome proliferator-activated receptor-gamma agonists inhibit experimental allergic encephalomyelitis by blocking IL-12 production, IL-12 signaling and Th1 differentiation. *Genes Immun* 2002; 3: 59–70.
- Naujoks-Manteuffel C, Niemann U. Microglial cells in the brain of *Pleurodeles waltl* (Urodela, Salamandridae) after wallerian degeneration in the primary visual system using *Bandeiraea simplicifolia* isolectin B4-cytochemistry. *Glia* 1994; 10: 101–13.
- Niino M, Iwabuchi K, Kikuchi S, et al. Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-gamma. *J Neuroimmunol* 2001; 116: 40–8.
- Parmentier-Batteur S, Bohme GA, Lerouet D, et al. Antisense oligodeoxynucleotide to inducible nitric oxide synthase protects against transient focal cerebral ischemia-induced brain injury. *J Cereb Blood Flow Metab* 2001; 21: 15–21.
- Patsouris D, Muller M, Kersten S. Peroxisome proliferator activated receptor ligands for the treatment of insulin resistance. *Curr Opin Invest Drugs* 2004; 5: 1045–50.
- Pompl PN, Ho L, Bianchi M, McManus T, Qin W, Pasinetti GM. A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. *FASEB J* 2003; 17: 725–7.
- Quinn J, Montine T, Morrow J, Woodward WR, Kulhanek D, Eckenstein F. Inflammation and cerebral amyloidosis are disconnected in an animal model of Alzheimer's disease. *J Neuroimmunol* 2003; 137: 32–41.
- Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998; 391: 79–82.
- Sagi SA, Weggen S, Eriksen J, Golde TE, Koo EH. The non-cyclooxygenase targets of non-steroidal anti-inflammatory drugs, lipoxygenases, peroxisome proliferator-activated receptor, inhibitor of kappa B kinase, and NF kappa B, do not reduce amyloid beta 42 production. *J Biol Chem* 2003; 278: 31825–30.
- Sastre M, Dewachter I, Landreth GE, et al. Nonsteroidal anti-inflammatory drugs and peroxisome proliferator-activated receptor-gamma agonists modulate immunostimulated processing of amyloid precursor protein through regulation of beta-secretase. *J Neurosci* 2003; 23: 9796–804.
- Shiomi T, Tsutsui H, Hayashidani S, et al. Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates left ventricular remodeling and failure after experimental myocardial infarction. *Circulation* 2002; 106: 3126–32.
- Stalder M, Phinney A, Probst A, Sommer B, Staufenbiel M, Jucker M. Association of microglia with amyloid plaques in brains of APP23 transgenic mice. *Am J Pathol* 1999; 154: 1673–84.
- Stewart WF, Kawas C, Corrada M, Metter EJ. Risk of Alzheimer's disease and duration of NSAID use. *Neurology* 1997; 48: 626–32.
- Sturchler-Pierrat C, Abramowski D, Duke M, et al. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci USA* 1997; 94: 13287–92.
- Tegeer I, Pfeilschifter J, Geisslinger G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. *FASEB J* 2001; 15: 2057–72.
- Van Dorpe J, Smeijers L, Dewachter I, et al. Prominent cerebral amyloid angiopathy in transgenic mice overexpressing the London mutant of human APP in neurons. *Am J Pathol* 2000; 157: 1283–98.
- Van Leuven F. Single and multiple transgenic mice as models for Alzheimer's disease. *Prog Neurobiol* 2000; 61: 305–12.
- Watson GS, Craft S. The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. *CNS Drugs* 2003; 17: 27–45.
- Weggen S, Eriksen JL, Das P, et al. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature* 2001; 414: 212–6.
- Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease—a double-edged sword. *Neuron* 2002; 35: 419–42.
- Xiang Z, Ho L, Yemul S, et al. Cyclooxygenase-2 promotes amyloid plaque deposition in a mouse model of Alzheimer's disease neuropathology. *Gene Expr* 2002; 10: 271–8.
- Yan Q, Zhang J, Liu H, et al. Anti-inflammatory drug therapy alters beta-amyloid processing and deposition in an animal model of Alzheimer's disease. *J Neurosci* 2003; 23: 7504–9.
- Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med* 2004; 351: 1106–18.
- Zhang F, Casey RM, Ross ME, Iadecola C. Aminoguanidine ameliorates and L-arginine worsens brain damage from intraluminal middle cerebral artery occlusion. *Stroke* 1996; 27: 317–23.