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BRIEF REPORT

Rosiglitazone Improves Spatial Memory and Decreases Insoluble $A\beta_{1-42}$ in APP/PS1 Mice

Julie-Ann O'Reilly · Marina Lynch

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Introduction

Identifying potential novel treatments for patients with Alzheimer's disease (AD) is a major challenge and drugs used for other indications, particularly if they have anti-inflammatory properties, are particularly attractive. The nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-activated transcription factor. PPARγ agonists are used in the treatment of type 2 diabetes, but have been shown to have anti-inflammatory effects which may contribute to their neuroprotective effects (Kapadia et al. 2008; Loane et al. 2009). For this reason, rosiglitazone has been investigated both in vitro and in vivo as a potential treatment for AD. Previous studies have shown that rosiglitazone improves spatial learning in Tg2576 mice (Pedersen et al. 2006) and reduces Aβ accumulation in 13-month-old J20 (Escribano et al. 2010).

On this basis, we evaluated the effect of rosiglitazone in modulating early pathological changes in 7-month-old APP/PS1 mice, a double transgenic model co-overexpressing amyloid precursor protein (APP) with the Swedish mutation and exon-9-deleted presenilin (PS1). Plaque deposition and increased $A\beta_{1-42}$ and $A\beta_{1-40}$ have been reported at 4 and 8 months in these mice (Garcia-Alloza et al. 2006); deficits in memory flexibility occur in 9-month-old mice (Filali et al. 2010).

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The data indicate that accumulation of Aβ in these mice was accompanied by a deficit in the reversal phase of learning in the Morris water maze and that treatment with rosiglitazone for 4 weeks attenuated these changes. This finding indicates that a brief treatment with rosiglitazone early in the pathological process may be beneficial.

Methods

Female wildtype and APPswe/PS1dE9 mice (Jackson Laboratories, USA) aged 7 months, maintained under controlled conditions (12-h light/dark schedule; 21–23°C) received maple syrup daily (50 μl; Newforge, Canada) with or without added rosiglitazone (rosiglitazone maleate; 6 mg/kg/day; Alpha Technologies, Ireland) for 2 weeks prior to behavioural testing and for 2 weeks during testing. Experiments were performed under license (Department of Health and Children (Ireland)) with ethical approval. Mice were assessed for their ability to find a perspex platform (diameter 15 cm) in the Morris water maze. A single habituation session was followed by 5 days of training (4× 1-min trials; 3 min inter-trial interval), a probe trial 24 h later, and a 5-day reversal training period after a further 24 h.

$A\beta_{1-42}$ and $A\beta_{1-40}$ was assessed using Multi-spot Aβ 3-plex plates (MesoScale Discovery, USA). Briefly, tissue was homogenized (SDS/NaCl, pH 10), centrifuged (15,000 rpm; 40 min; 4°C) and the supernatant sample containing soluble Aβ were neutralised (0.5 M Tris–HCl, pH 6.8; 10% *v*/*v*). Pellets containing insoluble Aβ were disrupted (23 kHz; 2× 30 s) in guanidine buffer (5 M guanidine–HCl in ddH_2O; Sigma, UK), incubated on ice (4 h), centrifuged (15,000 rpm; 30 min; 4°C), and equalised (0.4 mg/ml in guanidine buffer). Plates were blocked, washed, and detec-

tion antibody added according to the manufacturer's instructions, and samples or standards for $A\beta_{1-40}$ (0–10,000 pg/ml), and $A\beta_{1-42}$ (0–3,000 pg/ml) in 1% Blocker A solution were added, incubated (2 h; RT) and washed, and read buffer was added. The plate was read immediately using a Sector Imager plate reader and Aβ concentrations evaluated with reference to the standard curve.

Fixed brain sections were washed (PBS; 5 min), incubated in alkaline-saturated NaCl (NaOH; 1 M (2 ml) in NaCl (200 ml) 20 min; RT), incubated in alkaline Congo red solution (NaOH; 1 M (2 ml) in Congo red (200 ml; Sigma, UK); 30 min; RT), rinsed (dH_2O), incubated in methyl green solution (1% *w/v*; Sigma, UK; 30 s), washed (dH_2O) and dehydrated (95% ethanol, 100% ethanol, and 100% ethanol). Sections were dried and incubated in xylene (15 min). Coverslips were mounted using depex polystyrene (Electron Microscopy Sciences, USA), and dried (o/n). Congo red-positive Aβ plaques were counted in six representative sections from each animal and results were expressed as the number of plaques per section (10 μm).

Fixed and Triton X-100-permeabilized brain sections were incubated in blocking solution (10% NGS in 4% BSA in PHEM buffer (60 mM PIPES, 25 mM HEPES, 10 mM EGTA, 2 mM $MgCl_2$ up to 1 L with ddH_2O; pH 6.9); 2 h), and sequentially in rabbit anti-human anti-pan β amyloid$_{15-30}$ (0.834 μg/ml in PHEM containing 2% BSA and 5% NGS; Merck Chemicals Ltd, UK) or rat anti-mouse CD11b (20 μg/ml; AbD Serotec, UK; o/n). The secondary antibody was Alexa 488-conjugated goat anti-rabbit IgG (0.25 μg/ml in 2% BSA in PHEM with 5% NGS; 1.5 h; Biosciences, Ireland).

Cortical tissue was chopped, incubated for 30 min in PBS containing collagenase D (1 mg/ml; Roche Applied Science, Germany) and DNase I (10 μg/ml, Sigma, UK), filtered through a nylon mesh (40 μm) and centrifuged (1,200 rpm; 5 min; 20°C) to obtain a pellet; this was resuspended in cDMEM supplemented with 0.5 M sucrose and 10% *w/v* PEG-1,000. The resultant glia were incubated±$A\beta_{1-42}$ (8 μM), resuspended in FACS buffer (100 μl), centrifuged (1,200 rpm; 5 min; 20°C), washed and incubated in the presence of CD16/CD32 FcγRIII block (1:100 dilution; BD Pharmingen, USA; 10 min; RT). Cells were incubated with FACS antibodies (PE-labelled CD11b; 1:100; AbCam, UK; FITC-labelled IA/IE; 1:500; BD Pharmingen, USA; FITC-labelled CD80; 1:100; eBiosciences, UK) for 30 min in the dark at 4°C, washed and centrifuged (1,200 rpm; 5 min; 20°C) and assessed using flow cytometry (DAKO $CyAN_{ADP}$, Beckman Coulter, Ireland).

Results

Mean latency to find the platform and mean pathlength were decreased with time during the acquisition phase of the Morris water maze in all mice ($p<0.001$; ANOVA; $n=5$) and no treatment effect was observed (Fig. 1a, b). During the probe trial, there was no effect of genotype (39.96±5.51 vs 32.78±5.95; wildtype vs APP/PS1) or treatment (42.16±2.70 and 33.62±4.68; rosiglitazone-treated wildtype and APP/PS1 mice). No genotype- or treatment-related differences in swim speed were observed.

There was a significant time-related change in mean latency and mean pathlength ($p<0.01$; ANOVA) and a significant treatment effect in pathlength during the reversal phase of the task ($p<0.01$; Fig. 1c, d). Analysis of the data on day 5 revealed that mean latency and mean pathlength were significantly greater in APPswe/PS1dE9 mice than wildtype mice (**$p<0.01$; ***$p<0.001$; Fig. 1e, f) and that treatment with rosiglitazone significantly attenuated the changes observed in APPswe/PS1dE9 mice ($^{++}p<0.01$; $^{+++}p<0.001$; ANOVA).

Insoluble $A\beta_{1-42}$ concentration, but not of insoluble $A\beta_{1-40}$, (or soluble $A\beta_{1-40}$ and $A\beta_{1-42}$; not shown), was higher in tissue prepared from APPswe/PS1dE9, compared with wildtype mice (**$p<0.01$; ANOVA; $n=5$; Fig. 1g,h); this was significantly attenuated in rosiglitazone-treated APP/PS1 mice ($^{++}p<0.01$; ANOVA).

Congo red positive Aβ plaques were observed in the hippocampus or cortex of APPswe/PS1dE9 mice and rosiglitazone decreased plaque number in hippocampus, but not cortex ($^{++}p<0.01$; ANOVA; Fig. 2a,b). Marked Aβ-immunofluorescence (green) was observed in cortex and hippocampus of APP/PS1 mice and with evidence of CD11b-positive (red) staining, suggesting co-localization of activated microglia (Fig. 2c).

FACS analysis of acutely dissociated cells revealed that Aβ significantly increased the number of $CD11b^+$ cells prepared from wildtype mice which stained positively for CD80 or MHCII (***$p<0.001$; ANOVA) but that this effect was absent in cells prepared from wildtype mice which received rosiglitazone ($^{++}p<0.01$; see FACS plots (Fig. 2d) and Fig. 2e,f). The Aβ effect was absent in cells prepared from APP/PS1 mice and therefore a genotype-related effect of Aβ was identified ($^{\#}p<0.05$; $^{\#\#}p<0.01$). Analysis by two-way ANOVA indicated significant effects of genotype ($p<0.05$) and treatment ($p<0.05$) and interaction ($p<0.01$). The numbers of $CD11b^+$ $CD80^+$ cells and $CD11b^+$ IA/IE^+ cells (which were normalized to control values within each experiment) were increased by 47% and 250% respectively in APP/PS1 mice but these changes were not statistically significant.

Discussion

The data presented indicate that 7-month-old APP/PS1 mice exhibited plaque deposition and increased insoluble

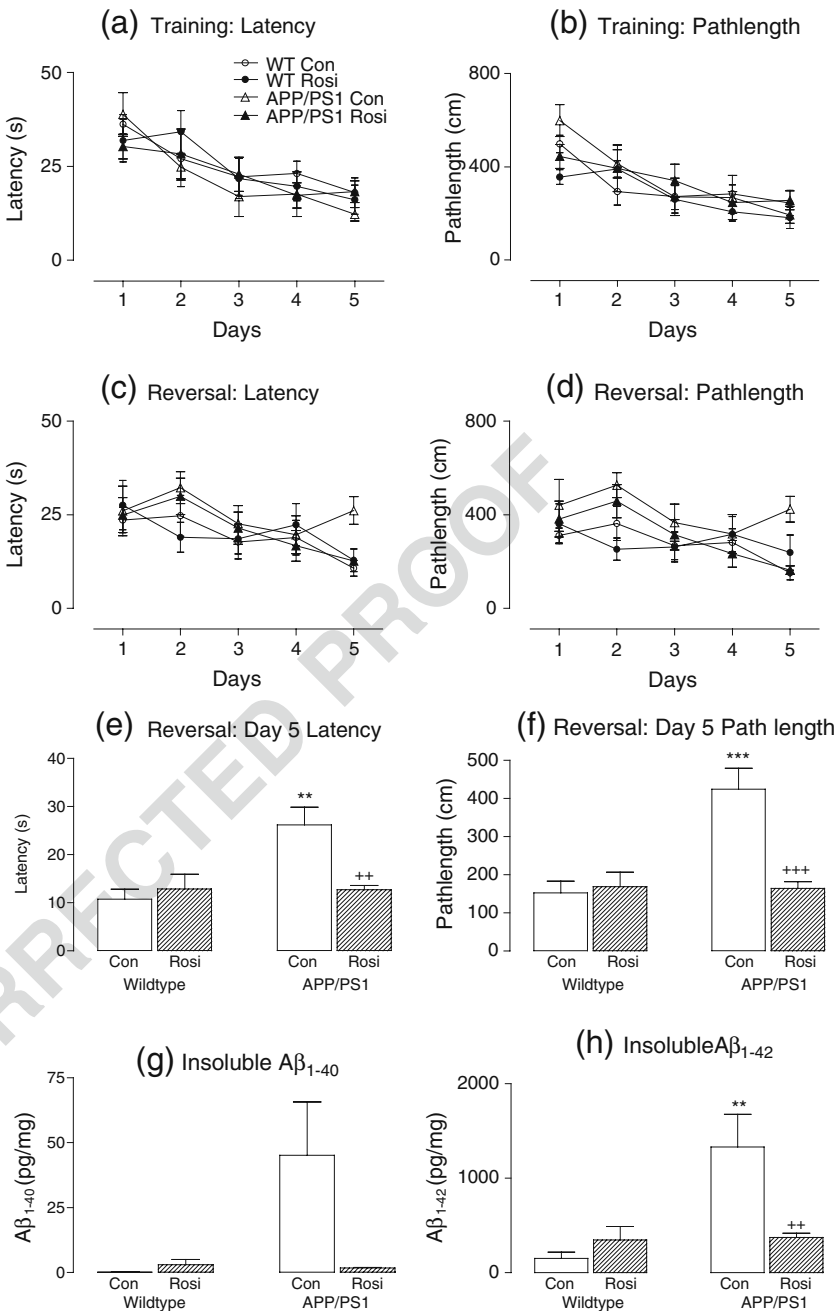
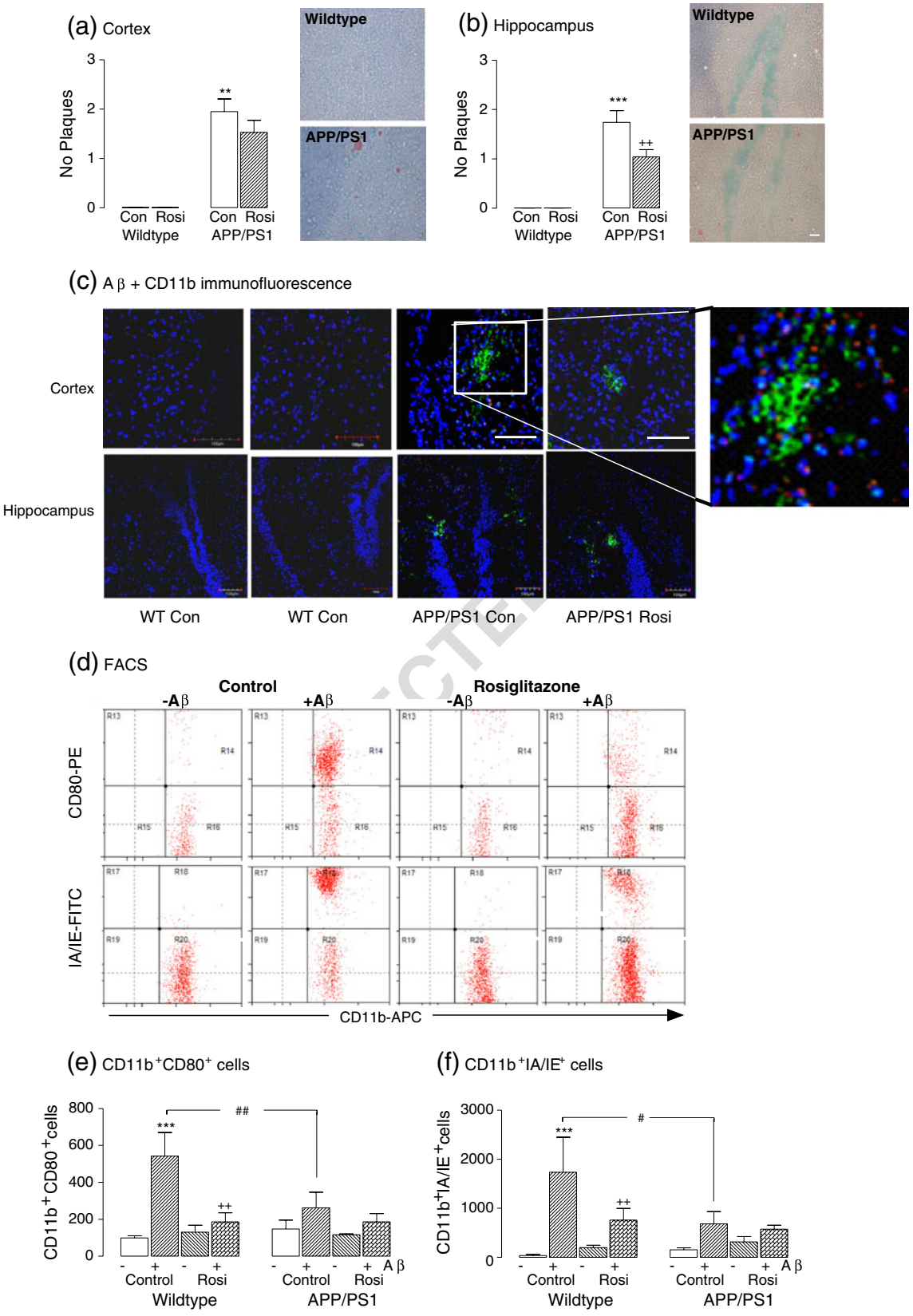


Fig. 1 Rosiglitazone attenuated the genotype-associated changes in spatial learning. Mean latency (**a**) and mean pathlength (**b**) were similar in the four groups of mice during acquisition, but there was a significant time-related change in mean latency (**c**) and mean pathlength (**d**) during the reversal phase ($p<0.01$; two-way ANOVA) and a significant treatment effect in pathlength ($p<0.01$). Analysis on day 5 (**e**,**f**) revealed that both were significantly greater in APP/PS1 mice than wildtype mice (**$p<0.01$; ***$p<0.001$); rosiglitazone significantly attenuated these changes ($^{++}p<0.01$; $^{+++}p<0.001$; ANOVA). Insoluble $A\beta_{1-42}$ (**h**), but not insoluble $A\beta_{1-40}$ (**g**), was increased in APP/PS1 mice (**$p<0.01$; ANOVA; $n=5$); rosiglitazone significantly attenuated this ($^{++}p<0.01$; ANOVA)

$A\beta_{1-42}$, accompanied by a deficit in the reversal, but not acquisition, phase of the Morris water maze. Rosiglitazone attenuated these changes. The lack of effect during acquisition has been reported in 9-month-old mice (Filali et al. 2010), although deficits in 12-month-old mice (Puolivali et al. 2002) and 14-month-old mice when performance reached an asymptote (Liu et al. 2002) have been described. Impairment in the probe test in 12-month-old mice has been correlated with Aβ accumulation (Puolivali et al. 2002), but this coupling was not observed here and may be age-sensitive. However learning flexibility, as assessed by behaviour in the reversal phase of the Morris water maze, was impaired in the present study and older APP/PS1 mice exhibited similar changes in the reversal learning phase in a T maze (Filali et al. 2010). Importantly, treatment of mice for 4 weeks with rosiglitazone attenuated the behavioural deficit, broadly agreeing with findings in J20 mice (which overexpress human APP with the Swedish and Indiana familial AD mutations) in which a 3-month treatment period was required to modulate changes in 13-month-old mice (Escribano et al. 2010). Similar treatment periods improved memory flexibility in 12-month-old APP/PS1 mice (Toledo and Inestrosa 2010) and 13-month-old Tg2576 mice (Pedersen et al. 2006).


(a) Cortex
No Plaques
Wildtype
APP/PS1
Con Rosi
Wildtype
Con Rosi
APP/PS1
(b) Hippocampus
(c) Aβ + CD11b immunofluorescence
Cortex
Hippocampus
WT Con
WT Con
APP/PS1 Con
APP/PS1 Rosi
(d) FACS
Control
Rosiglitazone
-Aβ
+Aβ
CD80-PE
IA/IE-FITC
CD11b-APC
(e) CD11b+CD80+ cells
CD11b+CD80+cells
(f) CD11b+IA/IE+ cells
CD11b+IA/IE+cells
Control
Rosi
Aβ

Q2

◀ **Fig. 2** Rosiglitazone decreased Aβ in APPswe/PS1dE9 mice. Congo red-positive Aβ plaques were increased in cortex (**a**) and hippocampus (**b**) of APP/PS1 mice (**$p<0.01$; ANOVA; $n=5$); this hippocampal change was attenuated in rosiglitazone-treated mice ($^{++}p<0.01$; ANOVA). **c** Aβ-immunofluorescence (*green*) was associated with $CD11b^+$ (*red*) staining in hippocampus (*see inset*) of APP/PS1 mice (*scale bars*, 100 μm). **d**–**f** $A\beta_{1-42}$ significantly increased expression of CD80, **e** and IA/IE, **f** on $CD11b^+$ cells isolated from wildtype, but not APPswe/PS1dE9, mice (***$p<0.001$; two-way ANOVA; $n=5$); a significant Aβ-induced genotype-associated change was observed ($^{\#}p<0.05$; $^{\#\#}p<0.01$). Rosiglitazone attenuated the Aβ-induced change ($^{++}p<0.01$; ANOVA)

Thus, the treatment period required to improve spatial learning appears to be age-dependent and therefore on the extent of the pathology.

APP/PS1 mice exhibited plaque deposition, decorated by $CD11b^+$ cells, and increased insoluble $A\beta_{1-42}$. Although plaques have been reported in 4-month-old animals, the earliest previous report of increased Aβ accumulation is 8 months (Garcia-Alloza et al. 2006). Rosiglitazone attenuated the genotype-related increase in Aβ, but its effect on plaques was less profound suggesting that a longer treatment period may be necessary to eliminate plaques in APP/PS1 mice as described in J20 mice (Escribano et al. 2010). Improved memory flexibility in rosiglitazone-treated APP/PS1 (Toledo and Inestrosa 2010) and J20 (Escribano et al. 2010) mice has been correlated with total Aβ, and the present findings broadly concur with these data.

Treatment of cells prepared from wildtype, but not APP/PS1, mice with Aβ increased microglial activation and this was decreased in rosiglitazone-treated mice supporting an anti-inflammatory role for rosiglitazone (Loane et al. 2009). This may be the mechanism by which rosiglitazone exerts its effects here since an inflammatory environment has been suggested to inhibit phagocytosis of Aβ (Koenigsknecht-Talboo and Landreth 2005) and also apoptotic cells (McArthur et al. 2010). Interestingly, cells prepared from APP/PS1 mice were refractory to added Aβ indicating that chronic exposure to Aβ alters microglial function, including phagocytic function allowing Aβ accumulation; however, rosiglitazone treatment for 4 weeks did not restore responsiveness of cells to Aβ. Thus, even very early pathological changes in APP/PS1 mice are affected differently by this rosiglitazone treatment regime, although significantly, this treatment was sufficient to reverse the behavioural deficit and reduce insoluble $A\beta_{1-42}$ in parallel.

Conflict of interest disclosures None

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