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BRAIN RESEARCH

Research Report

In vitro effect of PPAR-γ2 Pro12Ala polymorphism on the deposition of Alzheimer's amyloid-β peptides

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ABSTRACT

Mounting evidence suggests that peroxisome proliferator-activated receptor-γ (PPAR-γ) is involved in the modulation of pathogenic events related to Alzheimer's disease (AD). Such events would include the cerebral deposition of amyloid-β (Aβ) and the consequent local inflammatory response. PPAR-γ has been shown to act on both fronts, reducing either the secretion of Aβ or the expression of pro-inflammatory cytokines. Recently, the relatively common Pro12Ala polymorphism in exon 2 of PPAR-γ has been associated with higher risk for late onset AD. Here, we compare the effect of PPAR-γ and its genetic variant on the secretion of Aβ. Our results indicate that, in neuronal cultured cells, the Pro12Ala substitution does not affect the anti-amyloidogenic capacity of PPAR-γ. Additional factors, PPAR-γ related, may therefore predispose aged subjects, carrying the Ala allele, to develop the neurodegenerative disease.

1. Introduction

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a nuclear receptor with a key role in adipocyte differentiation. It is activated by certain fatty acids, in particular by nitrated fatty acids (Schopfer et al., 2005), prostanoids and thiazolidinediones (TZDs), a novel class of antidiabetic agents (Saltiel and Olefsky, 1996; Stumvoll and Haring, 2002). The PPAR-γ gene contains three promoters that yield three isoforms, namely PPAR-γ1, PPAR-γ2 and PPAR-γ3 (Fajas et al., 1997, 1998). PPAR-γ1 and γ3 RNA transcripts translate into the identical PPAR-γ1 protein. So far, a number of genetic variants have been identified in the PPAR-γ gene. These include two loss-of-function mutations (Val290Met and Pro467Leu) described in individuals with severe insulin resistance (Barroso et al., 1999), a rare gain-of-function mutation (Pro115Gln) associated with obesity (Ristow et al., 1998), the silent CAC478CAT mutation and the highly prevalent Pro12Ala polymorphism in PPAR-γ2 (Stumvoll and Haring, 2002; Yen et al., 1997).

Because body fat mass is a strong determinant of insulin sensitivity and PPAR-γ plays a key role in adipocyte differentiation, the influence of this nuclear receptor on susceptibility for type 2 diabetes has been largely investigated (reviewed in Lazar, 2005). In addition to its involvement in adipogenesis, PPAR-γ activation is associated with a reduction in the expression of several inflammatory genes (Jiang et al., 1998; Ricote et al., 1998). It was shown that, at high concentration, some non-steroidal anti-inflammatory drugs (NSAIDs) act as direct PPAR-γ ligands and, as a consequence, reduce cytokine production (Bishop-Bailey and Warner, 2003).

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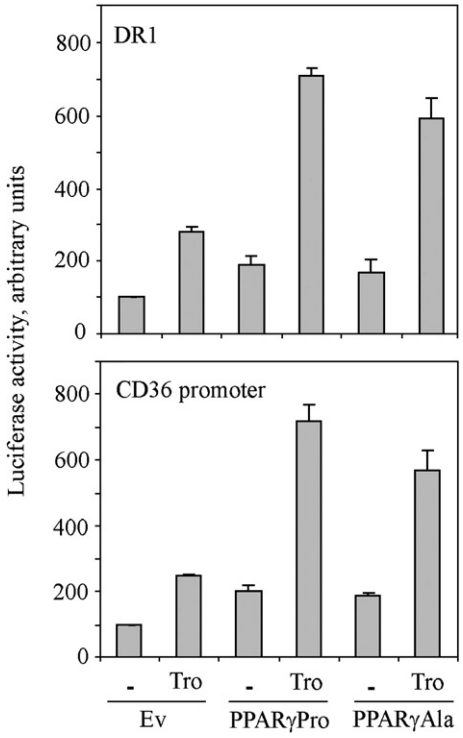

Fig. 1 – Transactivation capacity of PPAR-γ Pro and PPAR-γ Ala. Luciferase assay was performed, as described in the Experimental procedures section, on two PPAR-γ response elements: DR1 (upper panel) and a specific CD36 promoter region (lower panel). After 3 h transfection with PPAR-γ Pro, PPAR-γ Ala or with the empty vector (Ev), the cells were treated with 50 μM troglitazone (Tro) and harvested 24 h later. Results are expressed as the mean ± SD from three independent experiments.

Recently, a protective role of PPAR-γ against Alzheimer's disease (AD) has been suggested. Support for this hypothesis comes from two lines of evidence: (a) ibuprofen, indomethacin and naproxen are among the NSAIDs that potentially decrease the risk for AD (in t' Veld et al., 2001) and are proven to be effective PPAR-γ activators (Lehmann et al., 1997); (b) *in vitro*, PPAR-γ activation decreases the release of amyloid-β (Aβ), main component of the amyloid plaques associated with AD (Camacho et al., 2004; d'Abramo et al., 2005; Sastre et al., 2003). In line with these observations, a recent report suggests that the common PPAR-γ Pro12Ala polymorphism is associated with increased risk of developing late-onset AD (Scacchi et al., 2007).

In the present study, we compare the ability of PPAR-γ and its polymorphic variant Pro12Ala to decrease the release of Aβ in neuronal cultured cells. Our results indicate that release of both $A\beta_{1-40}$ and $A\beta_{1-42}$, the two amyloid species considered particularly relevant in AD, is significantly inhibited by either wild-type PPAR-γ Pro or its naturally occurring Pro12Ala variant.

2. Results

We compared by luciferase reporter assay the transactivation capacity of PPAR-γ wild-type (PPAR-γ Pro) and its polymorphic variant (PPAR-γ Ala) on two distinct PPAR-γ response elements (PPRE): DR1 and a specific human CD36 promoter region which is known to contain PPRE (Nagy et al., 1998; Ricciarelli et al., 2000). In both cases, as expected, the TZD troglitazone (50 μM) markedly increased the luciferase activity in cells transfected with the PPAR-γ Pro plasmid (Fig. 1). Although to a smaller extent, troglitazone was also able to increase the luciferase activity in cells overexpressing the Ala variant. These results agree with previously published works indicating a lower transactivation capacity of PPAR-γ Ala (Deeb et al., 1998; Masugi et al., 2000).

A number of studies have shown that, in cultured cells, overexpression of PPAR-γ reduces the secretion of Aβ (Sastre et al., 2003; d'Abramo et al., 2005). To test whether this phenomenon could be affected by the Pro12Ala substitution, we analyzed the secretion of total Aβ in mouse neuronal cells (N2a) stably transfected with the human amyloid precursor protein (APP695) and transiently transfected with the PPAR-γ Pro or Ala plasmid. The efficiency of transfections was monitored by immunoblotting (Fig. 2, upper panel). As expected, overexpression of PPAR-γ Pro remarkably decreased the total Aβ released in the culture medium; identical results, however, were obtained in the samples overexpressing PPAR-γ Ala (Fig. 2, lower panel). To further investigate this issue, the conditioned media were subjected to Aβ-specific ELISA to estimate the amount of secreted $A\beta_{1-40}$ and $A\beta_{1-42}$. Consistent with the results seen in immunoblotting, we found that cells overexpressing either PPAR-γ Pro or PPAR-γ Ala reduced the release of $A\beta_{1-40}$ to 60% of control cells. To a similar extent, both PPAR-γ constructs also reduced the secretion of $A\beta_{1-42}$ (Fig. 3).

3. Discussion

The Pro12Ala polymorphism in PPAR-γ represents the first genetic variant with a broad impact on the risk of common type 2 diabetes (Altshuler et al., 2000). It has been assumed that if the entire population carried the Ala allele, the prevalence for

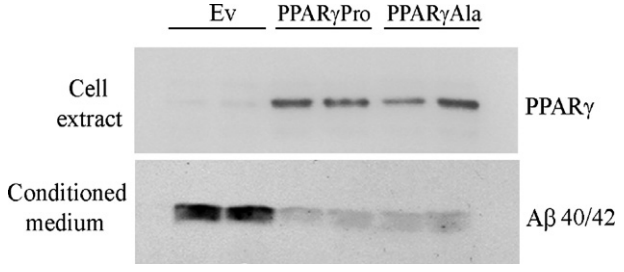

Fig. 2 – Effect of PPAR-γ Pro and PPAR-γ Ala overexpression on the secretion of total Aβ. N2a cells overexpressing human APP695 were transiently transfected with PPAR-γ Pro, PPAR-γ Ala or with the empty vector (Ev), as indicated. After 48 h transfection, cells were processed for immunoblot analysis of PPAR-γ (upper panel). For Aβ detection (lower panel), 30 μl of the collected media were electrophoresed on 16.5% Tris/Tricine gels, transferred onto nitrocellulose membranes and immunodetected with 6E10 antibody, reactive to amino acid residue 1–17 of human Aβ. The results shown are representative of three independent experiments with similar results.