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Research paper

The neuroprotective role of rosiglitazone in advanced glycation end product treated human neural stem cells is PPARgamma-dependent

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ABSTRACT

Hyperglycemia is accompanied by an accelerated formation rate of advanced glycation end products (AGEs), which is associated with the pathogenesis of diabetic neuronal deficits. Peroxisome proliferator-activated receptor gamma (PPARγ) belongs to a family of ligand-activated nuclear receptors and its ligands are known to control many physiological, pathological and inflammatory pathways. Weinvestigated the hypothesis that the PPARγ agonist (rosiglitazone) would abrogate AGEs-mediated neurotoxic effects on human neural stem cells (hNSCs), by whichAGEs may play a role in diabetic-related neuronal impairment. Here, we show that rosiglitazone treatment increases cell viability of hNSCs via downregulation of caspase 3 activity. These rescue effects were extended in our studies showingrosiglitazone-mediated activation of PPARγ reversed the expression levels of two neuroprotective factors (Bcl-2 and PGC1α) that were downregulated in hNSCs exposed to AGEs alone. The stimulation of mitochondrial function and anti-oxidative stress by rosiglitazone was associated with activation of the PGC1α pathway by up-regulation of mitochondrial (NRF-1 and Tfam) and oxidative defense (*SOD1*, *SOD2* and Gpx1) genes. Moreover, rosiglitazone significantly normalized the inflammatory responses (TNF-α and IL-1β), NF-κB (p65), and inflammatory genes (iNOS and COX-2) in the hNSCs treated with AGEs. This neuroprotective effect of *rosiglitazone* was effectively blocked by PPARγ-specific antagonist (GW9662), demonstrating that the action of *rosiglitazone* was mediated by at PPARγ-dependent pathway. Collectively, these novel findings show AGEs induce neurotoxic effects in hNSCs, and provide important mechanistic insights that may explain the increased risk of neuronal impairment deficits in diabetic patients. More importantly, these data show rosiglitazone-mediated activation of PPARγ-dependent signaling is neuroprotective in AGE-treated hNSCs, and suggests PPARγ ligands may be useful in the therapeutic management of patients with neurodegenerative diseases

1. Introduction

Diabetes mellitus (DM) is a prevalent disease characterized by an inability to produce, or respond to, insulin, leading to high blood glucose concentrations and ultimately, increased risk of tissue damage and disease including diabetic retinopathy, nephropathy and neuropathy. Type 2 DM (T2DM) shares similar characteristics with Alzheimer's disease (AD), in that it occurs more commonly in the elderly and increases morbidity (Ott et al., 1999; Pugazhenthi et al., 2016). The association between diabetes, dementia, cognitive decline and AD is still under active research and development, but several studies indicate that diabetes may be linked with an elevated risk of AD progression (Arvanitakis et al., 2004; Jayaraman and Pike, 2014; Vignini et al., 2013). The term 'type 3 diabetes' has also been suggested as synonymous with AD (de la Monte et al., 2006).

Experimental evidence suggests that levels of advanced glycation end products (AGEs) in the brain are elevated in cases of AD-related dementia compared to age-matched controls (Dei et al., 2002; Yan et al., 1994). Further, in diabetes and AD, evidence shows that neurodegeneration is worse than that seen in AD alone (Girones et al., 2004). The risk of dementia was also linked with the pathological changes of diabetes, such as chronic hyperglycemia and chronic hyperglycemia-mediated AGEs, and may be a major toxic effect of disturbed glucose metabolism (Ott et al., 1999; Strachan et al., 2011). AGEs are generated both intracellularly and extracellularly from a complex nonenzymatic reaction of reducing sugars and are highly reactive with

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macromolecules (Srikanth et al., 2011). Studies show AGEs stimulate Aβ aggregation and accumulation, and lead to plaque development (Vitek et al., 1994; Woltjer et al., 2003) and increased Aβ binding (Mruthinti et al., 2006). Moreover, several studies demonstrated that AGEs are directly neurotoxic (Takeuchi et al., 2000; Yan et al., 1994). Li and his collaborators showed AGEs induce tau hyperphosphorylation and impair synapses in SK-N-SH cells, primary hippocampal neurons, and rats (Li et al., 2012). Accumulation of AGEs is also one of the critical factors in the etiology of DM and its pathological complications, including AD associated neurodegeneration (Ramasamy et al., 2005; Srikanth et al., 2011). Taken together, these findings suggest T2DM and AD may share a common pathogenic mediator, namely AGEs (Janson et al., 2004; Ramasamy et al., 2005).

Impaired neurogenesis in the brain is correlated with the pathogenesis of AD and DM is linked to cognitive impairments and neurodegeneration (Ramos-Rodriguez et al., 2014; Verret et al., 2007). Because neurogenesis is essential for learning, memory and mood, there is a great research interest in defining the effects of neurodegeneration on the new neurons generated from neural stem cells (NSCs) (Jiang et al., 2008; Lee et al., 2015). As the name implies, NSCs are self-renewing, multipotent cells with unlimited proliferative potential, and differentiate as required during adulthood into the various phenotypes of the central nervous system, such as neurons, oligodendrocytes and astrocytes (Abrous et al., 2005; Gage, 2000). Recently we reported that hNSCs exposed to AGEs had significantly reduced cell viability, which correlated with increased activation of caspase 3 and 9 activities (Chung et al., 2015). This suggested the DM-mediated neurological dysfunction associated with AD may be due to impairment of NSC function (Girones et al., 2004; Ramos-Rodriguez et al., 2014).

*PPAR*γ is a crucial transcription factor for neuroprotection, antioxidative stress, anti-inflammation, lipogenesis and metabolism (Bordet et al., 2006; Kim et al., 2007; Lehrke and Lazar, 2005; Pseftogas et al., 2017; Qi et al., 2011; Sundararajan et al., 2006). PPARγ is also implicated in several brain diseases including stroke, AD, Parkinson's Disease (PD), Huntington's disease (HD), Multiple Sclerosis (MS) and Amyotrophic Lateral Sclerosis (ALS) (Bordet and Ouk, 2006; Chiang et al., 2010; Lehrke and Lazar, 2005; Sundararajan et al., 2006). Moreover, Wang and colleagues (2012) showed that the PPARγ agonist rosiglitazone enhances learning, place cell activity, and hippocampal synaptic plasticity in middle-aged rats via a glucose transporter-3 mechanism (Wang et al., 2012). Using an HD cellular model, we previously reported rosiglitazone had protective effects due to direct activation of PPARγ in striatal progenitor (ST14A) (Chiang and Chen, 2010), hepatoma (HepG2) (Chiang et al., 2011) and neuroblastoma cell lines (N2A) (Chiang et al., 2015; Chiang et al., 2012a). *We also showed* rosiglitazone down regulated TNFα effects in hNSCs (Chiang et al., 2013), which may prevent neuronal impairment. Recently, we also reported rosiglitazone-induced activation of PPARγ rescues Aβ-mediated toxicity in hNSCs, and show evidence supporting a neuroprotective role for activation of PPARγ signaling in Aβ-related diseases such as AD (Chiang et al., 2016b). PPARγ agonists are also known to regulate proliferation, differentiation and migration of NSCs (Morales-Garcia et al., 2011; Wada et al., 2006). Several studies reported rosiglitazone mediates AGE regulation of rat NSCs (Wang et al., 2009b) and proliferation through the BDNF–CREB pathway (Wang et al., 2011). Liang et al. demonstrated that rosiglitazone attenuates AGE induced dysfunction in endothelial progenitor cells (Liang et al., 2009). These data may provide intriguing clues about PPARγ stimulation on neurobiological events related to cellular function and disease. The potential connection between AGEs and PPARγ signaling in AD has not been explored widely. Here, the effects of rosiglitazone on AGE-induced damage in hNSCs were investigated to determine their protective capabilities.

2. Materials and methods

2.1. Cell culture

GIBCO® human neural stem cells (hNSCs) were originally obtained from National Institutes of Health (NIH) approved H9 (WA09) human embryonic stem cells. Complete StemPro® NSC serum free medium (SFM) was used for optimal growth and expansion of undifferentiated hNSCs as described previously (Chiang et al., 2012b). Complete StemPro® NSC SFM consists of KnockOut™ D-MEM/F-12 with 2% StemPro® Neural Supplement, 20 ng/ml of EGF, 20 ng/ml of bFGF, and 2 mM of GlutaMAX™-I.

2.2. Evaluation of cell growth

Cell viability was assayed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) absorbance and cell count as reported elsewhere (Chiang et al., 2012b). Synchronized hNSCs were treated as described with or without AGEs (BioVision, Milpitas, CA, USA) (0.5 mg/ml), PPARγ agonist rosiglitazone (10 μM) or PPARγ antagonist GW9662 (20 μM) (both from Cayman Chemicals, Ann Arbor, MI, USA) for 3 days. MTT solution (Sigma, Austin, TX, USA) was added to the culture medium for incubating the cells, and absorbance at 570 nm was measured in solubilized cells using an EZ Read 400 ELISA Reader (Biochrom, Holliston, USA). The cell growth rate was expressed as a percentage of values obtained in vehicle control.

2.3. Caspase activity assay

Caspase activity assay was carried out using caspase-3-like (DEVD-AFC) Fluorometric Protease Assay Kit (Chemicon, Michigan USA) as described previously (Chung, Chen, 2015). In brief, hemisected fresh cells were homogenized in the lysis buffer for 10 min. The cellular lysate (standardized to protein concentration) was incubated with an equal volume of 2 × reaction buffer (with 0.01 M of dithiothreitol) for an additional 1 h at 37 °C with caspase-3 substrates (DEVD-AFC) at a final concentration of 50 μM. Fluorescence was measured by a microplate reader with an excitation filter of 390 ± 22 nm and an emission filter of 510 ± 10 nm.

2.4. RNA isolation and quantitative real-time polymerase chain reaction (Q-PCR)

Total RNA was isolated and cDNA synthesis reactions were performed as described previously (Chianget al., 2016b). A real-time quantitative PCR was performed using a TaqMan kit (PE Applied Biosystems, Foster City, CA, USA) on a StepOne quantitative PCR machine (PE Applied Biosystems) using heat-activated TaqDNA polymerase (Amplitaq Gold; PE Applied Biosystems). The sequences of primers were as follows: PPARγ (5′-GACCTGAAACTTCAAGAGTACCAAA-3′ and 5′-TGAGGCTTATTG- TAGAGCTGAGTC-3′); Bcl-2 (5′-GGCTGGGATGC-CTTTGTG-3′ and 5′-CAGC- CAGGAGAAATCAAACAGA-3′); PGC1a (5′-TGAGAGGGCCAAGCAAAG-3′ and 5′- ATAAATCACACGGCGCTCTT-3′); NRF-1 (5′-CCATCTGGTGGCCTG- AAG-3′ and 5′-GTGCCTGGGTCCAT-GAAA-3′); NRF2 (5′-CAAGAACGCC- TTGGGATACC-3′ and 5′-AAACC-ACCCAATGCAGGACTT-3′); Tfam (5′-GAACAACTACCCATATTTAAAG-CTCA-3′ and 5′- GAATCAGGAAGTTCC- CTCCA-3′); SOD1 (5′-AAGGCCGTGTGCGTGCTGAA-3′ and 5′-CAGGTC- TCCAACATGCCTCT-3′); SOD2 (5′-GCACATTAACGCGCAGTCA-3′ and 5′-AGCCTCCAGCAA-CTCTCCTT −3′); Gpx1 (5′-CCTCAAGTACGTCCGACC-TG-3′ and 5′-C-AATGTCGTTGCGGCACACC-3′); iNOS (5′-GTTCTCAAGGCAC- AGGTC-TC-3′ and 5′-GCAGGTCACTTATGTCACTTATC-3′); COX2 (5′-ATCA-TTCACCAGGCAAATTGC −3′ and 5′- GGCTTCAGCATAAAGCGTTTG-3′); D-loop (5′-GGTTCTTACTTCAGGGCCATCA-3′ and 5′-GATTAGACC-CGTTACC- ATCGAGAT-3′); and GAPDH (5′-TGCACCACCAACTGCTT-AGC-3′ and 5′-GGCAT- GGACTGTGGTCATGAG-3′). Independent