REVIEW

CNS Neuroscience & Therapeutics

TGF-β1 Pathway as a New Target for Neuroprotection in Alzheimer's Disease

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Keywords
Alzheimer's disease; Apoptosis; β-amyloid; Cell cycle; Neurofibrillary tangles; Neuroprotection; Lithium; Transforming-growth-factor-β1.

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doi: 10.1111/j.1755-5949.2009.00115.x

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Alzheimer's disease (AD) is a neurodegenerative disorder that affects more than 37 million people worldwide. Current drugs for AD are only symptomatic, but do not interfere with the underlying pathogenic mechanisms of the disease. AD is characterized by the presence of ß-amyloid (Aβ) plaques, neurofibrillary tangles, and neuronal loss. The identification of the molecular determinants underlying AD pathogenesis is a fundamental step to design new disease-modifying drugs. Recently, a specific impairment of transforming-growth-factor-β1 (TGF-β1) signaling pathway has been demonstrated in AD brain. The deficiency of TGF-β1 signaling has been shown to increase both Aβ accumulation and Aβ-induced neurodegeneration in AD models. The loss of function of TGF-ß1 pathway seems also to contribute to tau pathology and neurofibrillary tangle formation. Growing evidence suggests a neuroprotective role for TGF-β1 against Aβ toxicity both *in vitro* and *in vivo* models of AD. Different drugs, such as lithium or group II mGlu receptor agonists are able to increase TGF-β1 levels in the central nervous system (CNS), and might be considered as new neuroprotective tools against Aβ-induced neurodegeneration. In the present review, we examine the evidence for a neuroprotective role of TGF-β1 in AD, and discuss the TGF-β1 signaling pathway as a new pharmacological target for the treatment of AD.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, affecting approximately 10% of individuals by 65 years of age and 47% by 85 years of age. It is mainly characterized by memory loss, with disoriented behavior and impairments in language, comprehension, and spatial skills also characterizing this disorder. Neuropsychiatric symptoms, such as agitation and psychosis are also frequent in people with AD, and are a common precipitant of institutional care [1]. The economic burden of AD is massive; in the United States alone, the annual cost care for patients with AD is approximately 150 billion of USD [2]. However, the number of therapeutic options for AD remains severely limited. Currently marketed drugs for AD (i.e., the acetylcholinesterase inhibitors, donepezil, rivastigmine, and galantamine, and the NMDA receptor antagonist, memantine) provides mainly symptomatic short-term benefit, without affecting the underlying pathogenic mechanisms [3]. Therefore, much effort is now directed to find treatments that effectively counteract the progression of AD. The comprehension of the molecular mechanisms underlying AD is therefore an essential step for the identification of new targets and the design of disease-modifying drugs able to slow down or even stop the degenerative processes and the resulting memory loss.

AD is characterized by the presence of extracellular aggregates of β-amyloid (Aß) in the senile plaques, intracellular aggregate of tau protein in the neurofibrillary tangles (NFT), and progressive neuronal loss. Different hypotheses have been proposed to understand the role of Aβ or tau protein in the pathophysiology of AD. The expression pattern of NFT in AD brain strongly correlates with the clinical onset and severity of dementia, but molecular genetics supports a primary role for Aß in the cascade of events leading to neuronal death in AD [4]. Oligomeric species composed of aggregated Aβ are believed to exert toxic effects on synaptic and cellular functions, finally leading to neurodegeneration.

In vitro studies have shown that Aß causes neuronal death via multiple mechanisms, which include membrane ion channel opening [5], radical oxygen species formation [6], amplification of NMDA toxicity [7,8], and cell cycle activation in differentiated neurons [9–13]. Furthermore, Aβ is known to promote the phosphorylation of tau protein and the subsequent formation of NFT through the activation of the two kinases, that is, the cyclin-dependent kinase 5 (CDK5) and the glycogen synthase kinase 3β (GSK-3β) [14–16]. In this scenario, tau hyperphosphorylation and NFT formation might be placed within the same molecular cascade initiated by Aβ, which leads to progressive synaptic loss, and, finally, to neuronal death.

In vivo studies have also been carried out for the analysis of neurotoxicity by Aß, but evidence is less consistent as compared to *in vitro* studies. Only a few transgenic mice overexpressing Aß show neuronal loss, perhaps because the lifespan of mice is too short for a full development of the death cascade [17]. Injection of Aß into the rodent brain causes a damage restricted to the injection site at best [18–20]. We can hypothesize that neurotoxicity of Aß *in vivo* is limited by the presence of endogenous protective factors that may be lacking in the AD brain [21–23]. One possible candidate is transforming-growth-factor β1 (TGF-β1). Transgenic mice lacking TGF-β1 show enhanced neuronal susceptibility to different neurotoxic insults [24].

In the present review, we examine the role of TGF-β1 in AD pathogenesis prior to discussing the rationale for considering TGF-β1signaling as a new target for neuroprotection in AD.

TGF-β1 Signaling Pathway: Smad and Non-Smad Dependent Pathways

TGF-β1 is a member of TGF-beta superfamily, which consists of several groups of highly conserved multifunctional cell–cell signaling proteins of key importance in the control of tissue homeostasis [25].

The TGF-β subfamily includes three isoforms in mammals, TGF-β1, 2, and 3, which are important modulators of cell survival, inflammation, and apoptosis [26], and also exert a central role in immune suppression, and repair after injury [27]. The three TGFβs are all synthesized as homodimeric proproteins (proTGFβ) that are around 400 amino acids in size and products of separate genes. The proTGFβs are cleaved intracellularly by furin into a larger C-terminal proregion also known as latency-associated peptide (LAP), and a shorter N-terminal active peptide, which forms the mature homodimers (25 kDa). LAP remains noncovalently associated with the mature TGFβ 25-kDa dimer before the complex is secreted [28]. The association between the TGF-β1, 2, and 3 prodomains (LAPs) and the corresponding mature growth factors prevents signaling through the TGF-β high affinity receptors [29]. Thus, TGF-bioactivity requires dissociation from LAP, a process termed latent TGF-β activation.

Extracellular activation of TGF-β is a critical but incompletely understood process *in vivo*. In particular, an important and unresolved issue in TGF-β biology regards the connection between matrix incorporation and activation of the latent TGF-β. A variety of molecules, from protons to different proteases, such as plasmin and trombospondin, have been described as latent TGFβ activators [30]. It seems that inactive TGF-β stored in tissues can be activated in response to injury and subsequent extracellular matrix perturbations. After TGF-β is released from its latency-associated peptide, it becomes able to initiate its diverse cellular responses by binding to, and activating specific cell surface receptors that have intrinsic serine/threonine kinase activity.

All three TGF-β isoforms interact with a high-affinity transmembrane receptor complex consisting of the activin-like kinase 5 (ALK5)/TGF-β type I receptor and the TGF-β type II receptor (TβRII) subunits [25] (see Figure 2). Several studies have demonstrated that ligand binding to TβRII induces the assembly of type I and type II receptors into complexes with the subsequent phosphorylation and activation of ALK5, which then propagates the signal inside the cell through the phosphorylation of receptor-regulated Smads (R-Smads: Smad2, Smad3, Smad5, and Smad8). The interaction between R-Smads and (ALK5)/TGF-β type I receptor is facilitated by the Smad anchor for receptor activation (SARA) [31]. Phosphorylated R-Smads form heteromeric complexes with Smad4. These complexes accumulate in the nucleus, where they regulate gene expression in a cell-type-specific and ligand dose-dependent manner through

interactions with transcription factors and specific promoter elements of target genes.

Smad6 and Smad7 are inhibitory Smads, which are known to counteract the signaling of R-Smads through different mechanisms [25]. Inhibitory Smads bind to activated type I receptors, thus inhibiting the phosphorylation and the following nuclear translocation of R-Smads. Furthermore, they can recruit E3-ubiquitin ligases targeting the receptor complex to the ubiquitin degradation pathway with the following inhibition of TGF-β/Smad signaling cascade.

Recent evidence suggests that TGF-β1 can also exert its biological effects through the activation of *smad-independent pathways* such as the extracellular-regulated kinase (ERK) pathways [32,33], the nuclear factor κB (NF-κB) pathway [34], and the phosphatidylinositol-3-kinase (PI3K)/Akt pathway [35].

Role of TGF-β1 in the Brain and in AD

In the central nervous system (CNS) TGF-β2 and 3 isoforms account for almost all the TGF-β immunoreactivity, while TGF-β1 expression has been found to be constitutive only in the meninges and choroid plexus and, most importantly, in some specific brain regions such as the hippocampus and the cortex [36]. Interestingly, TGF-β1 expression and release increase significantly in response to CNS lesions. Astrocytes and microglia seem to be the major sources of TGF-β1 in the injured brain [37], and several studies have shown that TGF-β1 induction during injury exerts a central role in preventing neurodegeneration [24,36].

An increased expression of TGF-β1 has been observed with age [37], and a protective role has been suggested for this neurotrophic factor in longevity [38]. Aging is characterized by an increased level of proinflammatory markers such as IL-6, TNF-α or IL-1β [38,39]. This state of subclinical chronic inflammation has been called "inflammageing," and seems to be involved in the pathogenesis of several age-related disorders such as cancer, diabetes, cardiovascular pathologies, and AD [39]. The protective role of TGF-β in aging and longevity has been suggested by *in vitro* and *in vivo* studies [40,41]. Increased plasma levels of bio-active TGF-β1 have been found in both male and female centenarians as compared to younger control subjects [41]. Similar results have been obtained by Forsey et al. [42] in octogenarian and nonagenarian subjects. Salvioli et al. [38] have also proposed that this age-related increase of TGF-β1 might counteract the proinflammatory status observed during aging, thus preventing the development of age-related disorders such as cancer and AD.

Changes in TGF-β1 serum and cerebrospinal fluid (CSF) levels have also been analyzed in AD. In particular, increased TGF-β1 levels have been found in CSF of AD patients [43,44], whereas a reduction of both its active (25 kDa) and inactive (50 kDa) forms has been reported in AD plasma [45].

Recently, a single nucleotide polymorphisms (SNPs) at codon +10 (T(C) and +25 (G/C) that affects the levels of expression of TGF-β1 has been associated with an increased conversion of mild cognitive impairment (MCI) in AD [46]. We have recently investigated the same polymorphism in healthy controls (HC) and AD patients. Preliminary data suggest that both the +10 C allele and the CC genotype are overrepresented in AD when compared to HC, and, that CC genotype might act as a risk factor for the development of late-onset AD (LOAD), independently of apolipoprotein status (unpublished results).

Many reports also describe a significant impairment of TGF-β1 signaling in AD brain [23,47–50]. The study by Tesseur et al. [48] strongly points to a causal role for of TGF-β signaling dysfunction in age-dependent neurodegeneration and AD pathogenesis (Figure 1). The authors found that the expression of TGF-β type II receptor (TβRII) by neurons is reduced very early in the course of AD, and this alteration seemed to be specific for AD and was not observed in other neurodegenerative conditions such as Parkinson's disease, frontotemporal dementia, or Lewy body dementia. The authors also found that a deficiency of TGF-β signaling, in a mouse model of AD, promoted both Aβ deposition and neuronal loss [48]. Moreover, Tesseur et al. [48] have shown that the

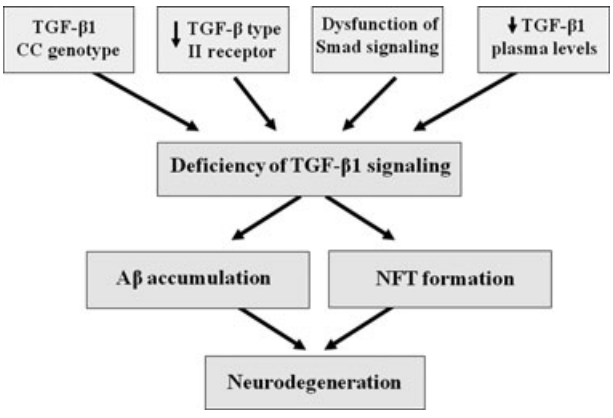

Figure 1 Hypothetical role of TGF-ß1 in AD pathogenesis. Alterations of TGF-β1 signaling in AD: (1) a reduced expression of the neuronal TGF-β type II receptor, (2) a dysfunction of Smad signaling, (3) a reduction in TGF-β1 plasma levels, (4) an increased occurrence of TGF-ß1 CC genotype which can promote the conversion of MCI into AD. All alterations might lead to Aβ accumulation and neurofibrillary tangles (NFT) formation with ensuing neurodegeneration.

impairment of TGF-β signaling in neuroblastoma cells resulted in neuritic dystrophy and increased levels of secreted Aβ and β-secretase-cleaved soluble amyloid precursor protein. These data suggest that a deficiency of TGF-β/TβRII signaling axis might exert a pathogenetic role in AD, depriving cortical neurons of trophic support, and finally promoting Aβ-induced neurodegeneration (see Figure 1).

However, the role of TGF-ß1 in AD pathophysiology is not unequivocal, and conflicting results have been reported recently. TGF-ß1 is known to induce the expression of the APP gene in several different cell culture systems [51,52] and might thus increase Aß production. The co-expression of TGF-ß1 in transgenic AD mice accelerates the deposition of Aß in cerebral blood vessels [53], and transgenic mice overexpressing TGF-ß1 develop AD-like vascular alterations [54]. In addition, vessel-derived TGF-ß1 has been suggested to contribute to inflammatory processes in the AD brain [55,56]. Town et al. [57] have recently found that blocking TGF-β-Smad 2/3 signaling reduces cerebrovascular β-amyloid deposits and Aβ abundance in Tg2576 mice, and these events result in promotion of Smad1/5/8 signaling with increased infiltration of Aβ-containing peripheral macrophages around cerebral vessels and β-amyloid plaques.

Overall data from the literature seem to suggest that TGF-ß1 can promote Aβ deposition in cerebral blood vessels, but reduces Aβ accumulation in the brain parenchyma [23]. In particular, it has been demonstrated that a modest increase in astroglial TGF-β1 production in aged transgenic mice expressing the human beta-amyloid precursor protein (hAPP) results in a 50% reduction of Aβ load in the hippocampus, and a decrease in the number of dystrophic neurites [58].

Deficiency of TGF-ß1 signaling is also involved in tau pathology and NFT formation. Luterman et al. [59] found that low levels of TGF-ß1 mRNA negatively correlated with NFT in the AD brain, thus suggesting that a deficiency of TGF-ß1 might also contribute to the cascade of events that result in the development of NFT-bearing neurons. The relationship between tau hyperphosphorylation and TGF-ß1 signaling has been recently studied in the temporal lobe in AD [50]. Interestingly, NFT can sequester phosphorylated Smad3 in AD brain, thus preventing its translocation into the nucleus and the induction of gene transcription [50].

Other groups report an impairment of Smad-dependent TGF-ß1 signaling in AD brain [47,49], with an aberrant localization of phosphorylated Smad2 to the cytoplasm rather than the nucleus of hippocampal neurons and a specific colocalization with amyloid plaques and NFT. These data suggest a dysfunction of Smad signaling in AD brain, and, interestingly, a recent *in vitro* study has demonstrated that Aβ can inhibit TGF-ß1 signaling by inducing the expression of Smad 7 [60].

Taken together, these data might explain the paradox observed in the AD brain, where TGF-ß1 levels in CSF are found to be high; however, this neurotrophic factor might not exert its neuroprotective action for an impairment of Smad signaling (Figure 1).

Smad proteins are also implicated in initiation and maintenance of neuronal differentiation and synaptic plasticity, and TGF-ß1 is a well-known inhibitor of cell proliferation that may contribute to keep postmitotic neurons in a differentiated state [32]. The reduced function of TGF-ß1 signaling in AD might therefore contribute to a re-expression of cell cycle proteins in neurons, and the resulting activation of the cell cycle, which is considered as an early event in AD pathogenesis [61–64].

We believe that a deficiency in TGF-ß1 signaling might exert a central role in AD pathogenesis via different mechanisms that finally lead to Aβ accumulation and/or NFT formation with an ensuing neurodegeneration (Figure 1).

Neuroprotective Effects of TGF-β1 against Aβ-Induced Neurodegeneration

TGF-ß1 is known to protect neurons against a diverse number of insults, including excitotoxicity, hypoxia, ischemia, and deprivation of trophic factors [23,36,65,66]. Several studies have suggested that TGF-ß1 also exerts a neuroprotective role against Aβ toxicity by selectively interfering with different steps of the Aß-induced death cascade. In cultured neurons, estrogen-stimulated release of TGF-ß1 from glial cells [67] or application of recombinant TGF-ß1 [68–71] reduces Aß-induced neurodegeneration.

We have recently studied the neuroprotective effects of endogenous TGF-β1 signaling in the rat brain after intracerebral injection of synthetic Aß [35]. Aß injection into the dorsal hippocampus produced only a small extent of neuronal loss in the pyramical layer of the CA1 region. However, Aß neurotoxicity was amplified by i.c.v. injection of SB431542, which behaves as a selective inhibitor of the activin-like kinase 5 (ALK5) TGF-β type I receptor [72].

Different molecular mechanisms have been implicated in the neuroprotective effects of TGF-β1 against Aß toxicity. TGF-β1 receptors are expressed both in glial cells and neurons [65], and, therefore TGF-β1 might exert its protective effects by acting on both cell types.

TGF-β1 has a constitutive role in the suppression of inflammation, and appears to control the degree of microglial activation in the CNS [24]. Inhibition of TGF-β1 in different models of neurodegenerative

disorders is associated with local inflammation mediated by macrophage/microglia and T cells [24,73]. Inflammatory responses elicited by elevated Aβ peptides play an important role in the progression of AD, and microglia activation is an early event in AD pathogenesis and can be already detected in patients with MCI [74]. Aß can activate microglia to release proinflammatory cytokines such as IL-1β, IL-6, and TNF-α [75], which can contribute to neuronal death in the AD brain. Interestingly, several studies have demonstrated that TGF-β1 reduces microglia activation and promotes the degradation of Aβ by the microglia [58,76,77].

TGF-β1 might also affect neuronal survival through other mechanisms because it acts synergistically with other neurotrophins and is required for a full neuroprotective activity of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial-derived neurotrophic factor (GDNF) [78–80]. The levels of BDNF and its receptor, tropomyosin receptor kinase B (TRKB), are reduced in the AD brain, and deficiency of BDNF signaling has been related to neurodegeneration and cognitive dysfunction in AD [81,82]. Interestingly, TGF-β1 enhances the expression of BDNF and TrkB in rat neuronal cultures [83].

It might be possible that the contemporary failure of both BDNF and TGF-β1 signaling in the AD brain enhances neuronal vulnerability to Aß, thus accelerating the progression of AD.

Finally, a component of the neuroprotective action of TGF-β1 is mediated by the activation of neuronal TGF-β receptors. TGF-β1 is known to prevent apoptotic cell death in neurons through the inhibition of caspase-3 activation [84]. In addition, TGF-β1 maintains mitochondrial membrane potential and increases the expression of antiapoptotic proteins, such as Bcl-2 and Bcl-xl [68]. TGF-β1 can also activate the extracellular-regulated kinase (ERK) pathway in hippocampal neurons, thus promoting the phosphorylation and subsequent inhibition of the proapoptotic protein, Bad [85]. Furthermore, TGF-β1 can increase the transcriptional activity of the antiapoptotic transcriptional factor, NF-kappaB, through the PI3K/Akt and ERK signaling pathways [86].

TGF-β1 Interferes with the Death Triggered by Aβ in Neurons: From Cell Cycle Inhibition to the Rescue of the Wnt Pathway

The process of neuronal death triggered by Aß proceeds along an aberrant re-activation of the cell cycle [10,11,13]. Cell cycle in proliferating cells depends on the sequential activation of cyclin (Cyc)/cyclin dependent protein kinases (CDKs), which control the transition through the different phases of the cycle [87]. Aß activates the cell cycle in neurons by inducing the sequential expression of different cell cycle proteins usually functioning in proliferating cells, such as cyclin D1, phosphorylated retinoblastoma protein (ppRB), cyclin E, and cyclin A, which are necessary for G1/S transition, and S phase progression [10]. Reactivation of the cell cycle is an obligatory step in the apoptotic pathway evoked by Aß, suggesting that an ectopic S phase triggers the signal for neuronal death. DNA replication has also been demonstrated in neurons from AD brains [88], providing the *in vivo* counterpart of *in vitro* findings.

Recent studies suggest that cell cycle activation in neurons leads to a pathological DNA replication performed by a noncanonical enzymatic machinery, which finally contributes to generation of a death signal in neurons [11,13]. As opposed to proliferating cells, neurons that enter the S phase in response to Aβ fail to express DNA polymerase-α (DNA pol-α), which has an essential role in the canonical DNA synthesis [11], but overexpress DNA polymerase-β (DNA pol-β), a repair enzyme that only occasionally performs *de novo* DNA synthesis. DNA pol-β is an error-prone enzyme and, therefore, the aberrant DNA synthesis induced by Aβ might contribute to the signal to trigger neuronal apoptosis. The extension of DNA replication performed by DNA pol-β is critical for the activation of a death signal that is mediated by an increased expression of p53, a major sensor of DNA damage in eukaryotic cells [11,12,13,89].

The increased expression of p53 in Aβ-treated neurons in response to DNA damage can promote neuronal death through different pathways. The activation of a p53/DNA damage-dependent pathway triggers the execution phase of apoptotic death via the induction of the proapoptotic protein Bax and the downregulation of the antiapoptotic protein Bcl-2 [90]. On the other hand, p53 induction in Aβ-treated neurons might also activate a slow degenerative process, which finally leads to NFT formation. Several genes are under the control of p53 in eukaryotic cells [89], and p53 has been shown to induce, in cultured neurons challenged with Aβ, the expression of Dickkopf (Dkk-1), a specific antagonist of the Wnt signaling pathway [91]. Wnt signaling has an established role in maintaining neuronal homeostasis, and GSK-3ß, the main enzyme involved in tau hyperphosphorylation [15], is a key component of the Wnt pathway. Activation of the Wnt pathway leads to the inhibition of GSK-3ß through a cascade of intracellular reactions, which involve adaptor proteins such as disheveled (Dvl), and a multiprotein complex containing GSK-3ß, β-catenin, axin, and adenomatous polyposis coli (APC) [92]. Inhibition of GSK3ß prevents tau phosphorylation and also

phosphorylation of ß-catenin, which thus escapes degradation and translocates to the nucleus where it drives the expression of different genes involved in the regulation of neuronal survival, such as Bcl-2 and survivin [93]. Accumulated β-catenin can also be targeted to synapses, where it modulates synaptic strength in response to depolarization [94,95].

In this scenario, the p53-dependent induction of Dkk-1 observed both in AD models and in the AD brain, may determine a loss of Wnt function, with the following activation of GSK-3ß and NFT formation [91,93]. Accordingly, Dkk-1 knockdown prevents tau hyperphosphorylation and β-catenin degradation in Aß-treated neurons [91].

Interestingly, we recently found that TGF-ß1 applied to cultured cortical neurons challenged with Aβ prevents the abnormal DNA replication, enhances the levels of Ser9-phosphorylated (inhibited) GSK-3β, and prevents β-catenin degradation and tau hyperphosphorylation, thus attenuating neuronal death. All these effects were abrogated by the PI3K inhibitor, LY294402, suggesting that TGF-ß1 is protective against Aβ neurotoxicity via the activation of the PI3K pathway (Figure 2). Interestingly, a defect in the PI3K pathway has been associated with AD [96,97]. Figure 2 also shows the classical Smad-dependent pathway leading to cell cycle inhibition in response to TGF-β. Whether this pathway contributes to cell cycle arrest and neuroprotection under conditions of a defective PI3K activation (as may occur in AD) is unknown.

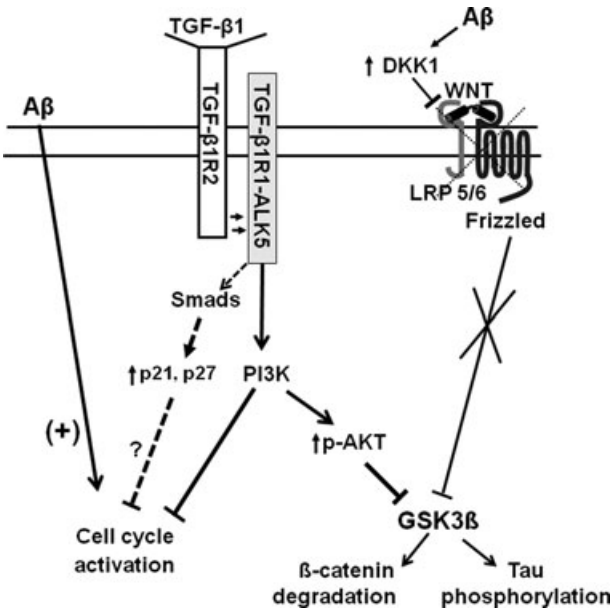

Figure 2 Putative mechanisms underlying the neuroprotective effects of TGF-β1 against Aβ-induced neurodegeneration. Aβ induces neuronal death via an early activation of cell cycle, and a late induction of DKK1 leading to an inhibition of the canonical Wnt pathway with ensuing activation of GSK-3β. TGF-β1 inhibits cell cycle activation and rescues the Wnt pathway via a direct activation of the PI3K pathway. Activation of the classical Smad-dependent pathway leading to an enhanced expression of cyclin-dependent kinase inhibitors (p21, p27) and cell cycle arrest is also shown (dotted). Whether this pathway may also contribute to the protective effect of TGF-β1 against Aβ-induced neurodegeneration is unknown.

Pharmacological Perspectives

The knowledge of the molecular processes underlying AD pathogenesis is a fundamental step for the development of disease-modifying drugs able to counteract the degenerative processes in AD.

According to the evidence discussed in the present review, the deficiency of TGF-ß1 signaling seems to be an early event in AD pathogenesis, which contributes to Aß-induced neurodegeneration and NFT formation in the AD brain (see Figure 1). We therefore suggest that the rescue of TGF-ß1 signaling might be a new strategy to promote neuroprotection in AD.

Neurotrophic factor therapy represents a difficult challenge for CNS drug discovery, because protein growth factors do not cross the blood–brain barrier and require intracerebral administration to be effective. The implant of autologous fibroblasts genetically modified to express human growth factors into selected areas of CNS has been proposed as a new strategy for the treatment of AD. This approach has been adopted for NGF in AD, and a phase I clinical trial has shown promising results [98]. Recently, BDNF gene delivery has also been found to exert substantial protective effects in AD models and has been proposed as a new potential therapy for AD [99]. However, central delivery of neurotrophic factors can be proposed only for a subset of AD patients and cannot be considered as a suitable approach for general medical practice.

The strong neuroprotective activity of neurotrophic factors has stimulated the search for small-molecules drugs that activate neurotrophic factor receptors or potentiate the action of growth factors by affecting their signaling pathway [100,101]. Along this line, small-molecule drugs that selectively activate specific elements of the TGF-β1 signaling pathway have been studied for the identification of drugs that can be beneficial in AD [102]. A more feasible approach would be the use of centrally available drugs that are able to increase the local production of TGF-ß1. Several drugs are known to induce the synthesis and the release of TGF-ß1 *in vitro* and *in vivo* (Table 1).

Estrogen treatment has been shown to reduce the risk of AD when administered at the time of the menopause

Table 1 Potential pharmacological approaches to rescue TGF-β1 signaling in AD

Drug	Mechanism	References
TGF-β1 mimetics	Activation of TGF-β1 receptors	[102]
Estrogens	Increased secretion of TGF-ß1 from astrocytes	[67]
Aspirin	Increased TGF-ß1 plasma levels *in vivo*	[111,113]
Statins	Stimulated TGF-ß1 synthesis and secretion from monocytes	[112,113]
Glatiramer	Induction of TGF-ß1 synthesis from Th2 type cells and glial cells	[116]
Sertraline, paroxetine	Increased TGF-ß1 plasma levels *in vivo*	[118,124]
Venlafaxine	Stimulated TGF-ß1 production and release from glial cells	[119]
Lithium	Increased release of TGF-ß1 from astrocytes	[See Figure 3]
mGlu2/3 receptor agonists	Increased synthesis and secretion of TGF-ß1 from astrocytes	[130,131]

and continued over several years [103]. Estrogens exert strong neuroprotective effects in hippocampal neurons when administered before Aβ treatment [104]. Interestingly, estrogens act as neuroprotectants via an increased secretion of TGF-ß1 from astrocytes [67]. TGF-ß1 released from astrocytes exposed to 17β-estradiol prevents Aß toxicity in pure neuronal cultures by preventing the unscheduled activation of the cell cycle [67]. These data suggest a possible role for TGF-ß1 in the neuroprotective activity of estrogen therapy in AD, but further evidence is needed to confirm this hypothesis.

Some cardiovascular drugs, such as aspirin and statins, can promote TGF-ß1 synthesis and release [105] and, interestingly, both of drugs are known to reduce the risk of developing AD [106–109]. Aspirin inhibits vascular smooth muscle cell proliferation via the TGF-ß1 pathway [110], and higher levels of active TGF-ß1 have been found in patients with coronary artery disease treated with aspirin [111]. Pravastatin has been found to increase TGF-ß1 synthesis and secretion in plaque monocytes from atherosclerotic patients [112]. In addition, the combination of atorvastatin with aspirin in patients undergoing coronary artery bypass grafting (CABG) has been shown to decrease the risk of major adverse cardiac events via the suppression of inflammatory responses and an increased production of TGF-ß1 [113]. Unfortunately, no studies have been carried out on the effects of these drugs on TGF-ß1 synthesis in the CNS.

Interestingly, some drugs used for the treatment of CNS disorders are known to promote TGF-ß1 synthesis in the brain. Glatiramer (GA) is a synthetic amino acid copolymer currently approved for the treatment of multiple sclerosis (MS) that reduces both relapse rate and progression of disability [114]. Different mechanisms of action have been postulated for this drug in humans. Arnon and Aharoni [115] have demonstrated that glatiramer in mice induces specific suppressor cells of the T helper (Th2) type that migrate to the brain where they express antiinflammatory cytokines such as IL-10 and TGF-ß1 in addition to BDNF [116]. Furthermore, GA-specific cells increase the expression of TGF-ß1 from glial cells in the cerebral cortex and hippocampus, two brain regions that are strongly implicated in the pathophysiology of AD. It could be interesting to examine the effects of glatiramer treatment on amyloid and tau pathology in AD models.

Different antidepressants, including tianeptine [117], sertraline [118], and venlafaxine [119], can increase TGF-ß1 production. Interestingly, therapeutic concentrations of venlafaxine prevent microglial activation, reduce proinflammatory cytokine secretion, and finally increase the release of TGF-ß1 in an astroglia–microglia coculture model [119]. These data suggest that glial cells can mediate the antiinflammatory effects of antidepressant drugs, but the potential neuroprotective activity of these compounds has been only partially explored in AD models. Presymptomatic treatment with the antidepressant paroxetine reduces both amyloid and tau pathology and also reverses memory impairment in the 3xTgAD mouse model of AD [120]. This study suggests that antidepressants are neuroprotective activity and may retard the development of AD. Accordingly, a history of major depression early in life has been considered as a risk factor for later development of AD [121]. Furthermore, the presence of depressive symptoms significantly increases the conversion of MCI into AD [122]. Plasma TGF-ß1 levels are reduced in major depressed patients and show a significant negative correlation with the Hamilton Depression Rating Scale (HDRS) [123]. Interestingly, different antidepressant drugs, including venlafaxine, paroxetine, and sertraline, significantly increase circulating TGF-ß1 levels in major depressed patients [118,124]. A recent study by Kessing et al. (2009) shows that continued long-term antidepressants treatment is associated with a reduction in the rate of AD [125], suggesting that it might be worth to assess whether TGF-β1 signaling is a common target for both depression and AD.

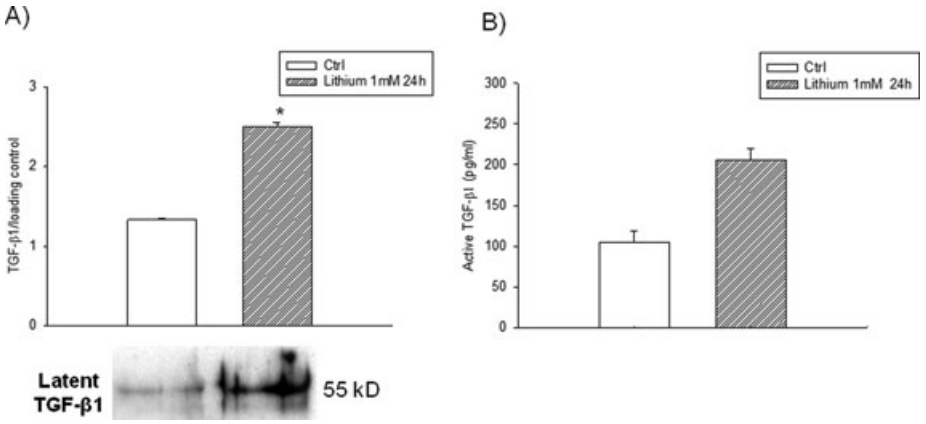

Figure 3 Lithium stimulates TGF-β1 release from rat cortical astrocytes. Rat cortical astrocytes were exposed to 1 mM lithium chloride for 24 h, and the incubation medium was collected for western blot analysis (A) and ELISA assay (B). (A) Representative immunoblot of latent TGF-β1 (about 55 kDa). Protein loading was checked by staining membrane-transferred proteins with Ponceau's solution. Values are the means ± SEM of 3 determinations; $P < 0.05$ (by one-way ANOVA + Fisher's LSD test) versus control (*). (B) Each bar represents the mean ± SEM of active TGF-β1 protein levels in the incubation medium. Data are from three different experiments; $P <$ 0.05 (by one-way ANOVA + Fisher's LSD test) versus control (*).

Other psychotropic drugs such as lithium ions can also influence TGF-ß1 production. Recent evidence suggests that lithium is neuroprotective against a variety of neurodegenerative conditions, including AD [126]. Prevalence of AD is lower in patients treated with lithium [127], and lithium reduces the risk of developing AD in elderly patients with bipolar disorder [128]. Different molecular mechanisms have been suggested to explain the neuroprotective effects of lithium in AD, such as the reduction of Aβ production or, more important, the inhibition of GSK-3β, which might counteract the loss of Wnt signaling observed in the AD brain [35,126]. Recently, we have found that lithium strongly induces the release of TGF-ß1 from rat cortical astrocytes, as assessed by immunoblotting and ELISA assay (unpublished results; see Figure 3). Hence, our own data suggest that the very broad neuroprotective activity of lithium might be related to the induced release of TGF-ß1 from glial cells.

Additional levels of interaction between TGF-ß1 signaling and lithium-regulated pathways have been proposed, including lithium's inhibition of Smad3/4-dependent TGF-ß1 signaling in neurons [129]. This inhibition of Smad-regulated gene transcription seems to be useful under specific pathological conditions (e.g., mood disorders) that may benefit from the suppression of plasminogen activator inhibitor type-1 (PAI-1) transcription [129]. The evidence that lithium's inhibitory effects on Smad3/4 transcriptional activity are due, at least in part, to the activation of PI3-K/AKT signaling [129], suggests that in neurons the activation of the PI3-K/AKT pathway might be mutually exclusive with the activation of the Smad-dependent pathway. In other words, the activation of Smad/non-Smad signaling pathways by TGF-ß1 might be fine-tuned in a context-dependent manner to result unchangingly into neuroprotection.

Finally, the production of TGF-ß is enhanced by agonists of group II metabotropic glutamate (mGlu) receptors both in cultured astrocytes [130] and in the mouse brain [131]. In addition, these drugs protect neurons grown in the presence of astrocytes against Aß toxicity [132]. It is noteworthy that highly potent and centrally available group II mGlu receptor agonists, such as LY404039, are under clinical development for the treatment of schizophrenia [133]. We suggest that these drugs are potential candidates as neuroprotectant agents in AD, and might be also useful for the treatment of psychosis in AD (PAD) as an alternative to antipsychotic drugs, which may increase the risk of cerebrovascular events in demented patients [134].

Acknowledgments

This work was supported by PRIN 2007 from the Italian Ministry of University and Research, and by Progetto MUR 105/04.

Conflict of Interest

All authors declare that no potential conflict of interest exists, including all relevant financial interests in any company or institution that might benefit from the publication.

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