J Exp Clin Med 2012;4(1):1–7

Contents lists available at SciVerse ScienceDirect

Journal of Experimental and Clinical Medicine

journal homepage: http://www.jecm-online.com

REVIEW ARTICLE

Modes of Action of Taurine and Granulocyte Colony-stimulating Factor in Neuroprotection

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ARTICLE INFO

Article history:
Received: Sep 22, 2011
Accepted: Oct 19, 2011

KEY WORDS:
apoptosis;
G-CSF;
G-CSF receptors;
neuroprotection;
taurine;
taurine receptors

New therapeutic targets are becoming increasingly popular for the treatment of a wide array of neurodegenerative diseases, the preferred targets being those that prevent neuronal apoptosis at multiple levels or those that can cross the blood-brain barrier in order to replace degenerated cells and promote neuronal regeneration. One such rapidly emerging neuroprotective agents is taurine. Taurine is a ubiquitous amino acid that satisfies most criteria to be classified as a neurotransmitter. Because of a wide spectrum of effects that taurine can induce on intrinsic apoptosis pathways, such as modulating mitochondrial pore permeability, attenuating endoplasmic reticulum stress, maintaining calcium homeostasis, and downregulating the activities of a range of pro-apoptotic proteins, including calpain and caspases, while upregulating a variety of anti-apoptotic proteins involved in glutamate and hypoxia-induced toxicity, taurine is being extensively studied and successfully applied for the treatment of neurodegenerative diseases. Another potential molecule being researched for combating neurodegenerative diseases is granulocyte colony-stimulating factor (G-CSF), which originates from the cytokine family of growth factors. G-CSF has gained widespread attention because of its ability to cross the blood-brain barrier, the presence of its receptors in the central nervous system, anti-apoptotic functions, and its proliferative role in the restoration of tissue survival via neurogenesis. In this review from the available current literature, the modes of action of taurine and G-CSF are discussed. Further mechanistic studies are warranted in order to fully realize the potential of these two molecules.

1. Introduction

Taurine (2-amino-ethanesulfonic acid) is one of the most abundant amino acids in the human body. The biosynthesis of taurine is believed to be incomplete in astrocytes and neurons, but metabolic cooperation between these two cell types is essential for the completion of its metabolic pathway.[1] Taurine is ubiquitously distributed, but is enriched in electrically excitable tissues such as the brain, retina, heart, and skeletal muscles.[2] The regulatory role of taurine has been implicated in a plethora of functions such as an anti-inflammatory molecule,[3,4] osmolyte, anti-oxidant,[2,5,6] trophic factor,[7,8] and as a neuromodulator.[9–11] Clinically, taurine has been used with varying degrees of success for the treatment of a variety of conditions, including, but not limited to, cardiovascular diseases, hypercholesterolemia, epilepsy, macular degeneration, Alzheimer's disease, hepatic disorders, alcoholism, cystic fibrosis, and, most recently in *in vitro* fertilization.[12,13]

Although taurine is not fully recognized as a neurotransmitter, it satisfies most of the criteria necessary to be classified as one. Co-localization of the taurine-synthesizing enzyme, cysteine sulfinic acid decarboxylase (CSAD), and taurine on the presynaptic side of a nerve has been documented, particularly in association with synaptic vesicles.[14–16] Interestingly, taurine is the only free amino acid that is highly enriched in the synaptic vesicles in comparison with glutamic acid, glutamine, gamma-amino butyric acid (GABA), and aspartic acid, which are also available in the synaptic vesicle fractions.[17] Taurine release is attributed to depolarization-evoked, calcium-dependent pathways and sodium-dependent, calcium-independent pathways under very high potassium concentrations.[18,19] About two decades ago, we demonstrated the presence of unique taurine receptors, and additional studies have reported the kinetic properties of highly specific taurine receptors that are neither agonists nor antagonists of structurally related amino acids such as glutamate, GABA, or glycine-activated taurine receptors.[20] Recently, an independent report further strengthened our findings that a specific recognition site exists that is used exclusively by taurine.[21] Taurine is known for its ability to neuromodularly inhibit postsynaptic taurine receptors and act as an indirect agonist of GABAA and glycine receptors, thereby increasing the duration of chloride channel conductance.[22]

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1878-3317/$ – see front matter
doi:10.1016/j.jecm.2011.11.001

Apart from this, the presence of a sodium-dependent taurine transporter (TauT) has been confirmed, and TauT knockouts demonstrate retinal degeneration, reduced olfactory sensitivity, and the manifestation of clinically important age-dependent diseases.[23–25] It is widely accepted that a biochemical mechanism is required to clear a neurotransmitter from the synaptic cleft after neurotransmission in order to maintain levels below toxicity. For taurine, plasma membrane transporters that are involved in the uptake of taurine from different brain regions, such as the cerebellar regions, the hypothalamus, and neuroglia, have been reported.[26–28] Although taurine meets the above mentioned criteria, it has been suggested that the presence of a vesicular taurine transporter and the process of vesicular membrane uptake of newly synthesized taurine to be loaded into taurinergic synaptic vesicles clearly defines taurine as a neurotransmitter and, hence, the acceptance of the theory of a taurinergic phenotype. No such evidence supporting the presence of a vesicular taurine transporter or the vesicular uptake of taurine has been documented. In fact, it has been confirmed that aspartate, taurine, and proline are not taken up by any synaptic vesicle, unlike similar amino acids such as glutamate, GABA, and glycine.[29] More specific studies are required to examine the proposed putative role of taurine in the central nervous system (CNS). So far, we have examined pertinent information related to the ways in which taurine exerts its neuroprotective effects, and these findings are presented in this review.

Both regenerative medicine and tissue engineering have great potential in clinical medicine because they can completely replace damaged tissue and promote the proliferation and differentiation of terminal cells that cannot otherwise be revived. The cells of the nervous system were once thought to be incapable of regeneration. However, with the success of therapeutic strategies involving the intervention of potent growth factors or cytokines, new cells can be propagated from progenitor cells. For neurons, growth factors such as granulocyte colony-stimulating factor (G-CSF), stromal cell-derived factor-1 (SDF-1), brain-derived neurotrophic factor (BDNF), and glial-derived neurotrophic factor (GDNF) have become increasingly popular for the treatment of a wide spectrum of neurological diseases, including Parkinson's disease, Huntington's disease, neuropathic pain, stroke, etc.[30–32] Newer reports suggest that G-CSF plays a role in memory impairment in senescence-accelerated mice.[33] G-CSF has been approved by the Food and Drug Administration for clinical use in patients with neutropenia and cancer patients receiving bone marrow transplant, in addition to being used as a novel drug for treating stroke patients.[34] G-CSF and its receptors are widely expressed in the neurons of the CNS and, more importantly, in adult neural stem cells.[35] Interestingly, G-CSF is able to steadily pass through the blood-brain barrier in intact rats, as demonstrated by a study that utilized G-CSF-iodine dye.[36] G-CSF protects against a number of neurological diseases, such as Parkinson's disease,[32] Huntington's disease,[37] and cerebral ischemia.[38] G-CSF stimulates the neural progenitor response *in vivo* and markedly improves long-term behavioral outcomes after cortical ischemia.[31] Peripheral infusion of G-CSF enhances the recruitment of progenitor cells from the lateral ventricle wall into ischemic areas of the neocortex in rats.[31] In this review, the molecular mechanisms by which G-CSF contributes to neuroprotection will be discussed.

2. Mechanisms of action of taurine in neuroprotection

2.1. Neuromodulatory role of taurine in the maintenance of intracellular calcium homeostasis

Although normal calcium signaling is crucial for normal physiological functions, calcium dyshomeostasis is a major event in the pathophysiology of a plethora of neurological diseases, including Alzheimer's disease, cerebral ischemia, Huntington's disease, etc.[39–41] Cells are endowed with calcium-permeable membrane receptors and channels that are voltage- or ligand-gated, or lodged with ATP-driven pumps such as Na^+/Ca^{2+} exchangers and plasma membrane Ca^{2+} ATPase, which collectively maintain low levels of intracellular calcium. Excessive activation of glutamate receptors is known to cause a heavy influx and accumulation of calcium inside the cell and is considered as one of the routes that ultimately results in neuronal death. This is mediated by excessive glutamate release because failure to re-uptake calcium by neurons and astroglia has been linked to CNS insults such as traumatic brain injury and Parkinson's disease, to name a couple.[42] We and other researchers have shown that taurine exerts its protective effects on neurons by effectively regulating intracellular calcium levels. It was initially shown that taurine protects against glutamate-induced neuronal damage by inhibiting the reverse mode of Na^+-Ca^{2+} exchangers.[9–11] Further studies have indicated that the protective effects of taurine are also facilitated through L-, P/Q-, and N-type voltage-gated calcium channels and N-Methyl-D-aspartic acid (NMDA) receptors.[43] Taurine is also implicated in the inhibition of glutamate-induced release of calcium from internal pools.[44]

2.2. Prevention of glutamate-induced apoptosis by taurine

Glutamatergic neurotransmission is at center stage in neuronal development, differentiation, migration, survival, learning, and memory formation.[45,46] However, a high concentration of glutamate is associated with the clinical characteristics of various diseases, including stroke, brain trauma, Parkinson's disease, etc.[42,47] We have reported that taurine prevents glutamate-induced activation of calpain and caspase-9 in rat primary neuronal cultures.[48] In addition, pre-incubation with taurine prior to glutamate treatment markedly reduced the number of apoptotic cells, as indicated by Hoechst staining, lowered the Bax/Bcl-2 ratio, and attenuated intracellular Ca^{2+} levels.[9,10,48] A gerbil model of transient focal cerebral ischemia designed to detect alterations in amino acids revealed significant elevations of GABA and taurine, perhaps to combat the surge in postischemic glutamate.[49–51]

2.3. Taurine downregulates key players in the intrinsic apoptosis pathway

Taurine is a strong modulator of apoptosis and is widely known to prevent elevated levels of caspases, calpains, and pro-apoptotic proteins such as Bad, Bax, and Bim. Taurine has been reported to significantly reduce apoptotic death by downregulating the activities of caspase-3 and intracellular calcium.[52,53] Taurine also represses ischemia-induced caspase-8 and caspase-9 expression in mouse hypothalamic nuclei.[54] Not only does taurine exercise its anti-apoptotic effects by inhibiting the activation of caspases, but it has also been shown to synergistically upregulate calpastatin while downregulating calpain in a model of focal cerebral ischemia.[55] A taurine-conjugated form of tauroursodeoxycholic acid (TUDCA) has been shown to be more beneficial than taurine for cell protection. TUDCA reduces the apoptotic threshold induced by glutamate in rat cortical neurons by causing phosphorylation and translocation of Bad from the mitochondria to the cytosol, which is the primary step in inactivating the release of cytochrome c from the mitochondria and triggering the activation of the caspase cascade. TUDCA also appears to modulate, in part, the activation of the PI3 K-dependent Bad signaling pathway.[56] This also appears to be true in an Alzheimer's disease model of amyloid-beta-induced pathogenesis.[57] TUDCA has been successfully applied to combat apoptosis-induced Parkinson's and Huntington's disease models.[58,59]

The cytoprotective role of taurine has been extended to preserving the integrity of mitochondrial pore permeability. Mitochondrial dysfunctions have deleterious consequences on neurons via the increased production of reactive oxygen species (ROS), ATP depletion, and the activation of cell death processes. Based on the current literature, it is apparent that taurine protects against hypoxia-induced apoptosis by preventing mitochondrial dysfunction.[60] Calcium overload and ionic imbalances in neurons induce mitochondria to produce free radicals.[61,62] Elevated levels of ROS is a hallmark of neurodegenerative diseases, especially Parkinson's disease.[63] Both taurine and TUDCA have been implicated in the significant inhibition of mitochondrial membrane alterations and antagonizing glutamate- and chemical hypoxia-induced calcium overload.[64–67] Direct evidence supporting taurine's ability to block mitochondrion-mediated cell pathways has been published.[68] Disruption of the mitochondrial respiration chain leads to cellular swelling followed by osmolyte efflux, as shown by the pathology of stroke.[69–71] Taurine is known to enhance cell volume regulation when neurons are swollen under extreme pathological conditions.[72] It has also been shown that taurine efficiently reduces cellular swelling following exposure to oxygen-glucose deprivation and reoxygenation-induced damage in rat brain cortical slices.[73]

When misfolded or unfolded proteins queue up in the endoplasmic reticulum (ER), the unfolded protein response (UPR) is generated, which then stalls protein synthesis until the proper fold-enhancing molecules are gathered. If the cell is unable to take the quanta of mis- or unfolded proteins, then UPR triggers the caspase-12-mediated apoptotic pathway, which operates exclusively in the ER.[74] UPR is mediated by ER transmembrane receptor-activating transcription factor 6 (ATF6), inositol-requiring kinase 1 (IRE1), and double-stranded RNA-activated protein kinase 1 (PKR)-like endoplasmic reticulum kinase (PERK). ER stress is known to be manifested in a variety of brain diseases like Alzheimer's disease, Huntington's chorea, Parkinson's disease, and amyotrophic lateral sclerosis.[75,76] It is reasonable to believe that cross-talk exists between mitochondria and the ER via the caspase cascade and aberrant calcium signaling. In fact, the synergistic actions of mitochondrial dysfunction and ER stress are both responsible for the pathophysiology of variety of diseases.[77,78] Our recently published data indicate that taurine protects against glutamate-induced excitotoxicity in primary cortical neurons and hypoxia-induced toxicity in PC12 cells by downregulating the expression of CHOP, GRP78, Bim, and caspase-12, which are the key proteins related to ER stress.[79,80]

2.4. Taurine counteracts excitotoxic upsurges by interacting with GABAA and glycine receptors, thereby increasing the duration of chloride conductance

Several lines of evidence indicate that taurine inhibits neurotransmission by binding to ionotropic GABAA and glycine receptors; this has been effectively used for the treatment of Alzheimer's disease.[22,81] Taurine conducts the flow of chloride not by increasing the frequency of the opening of the chloride channels, but by increasing the duration that the channel is open.[82] Very few studies have been conducted on the direct activation of taurine receptors. Definitive identification of taurine receptors is still emerging.[20,21] Further mechanistic studies are necessary to understand the direct role of taurine receptors on propagating neurotransmission. A summary schematic depicting the mode of action of taurine as a neuroprotective agent is shown in Figure 1.

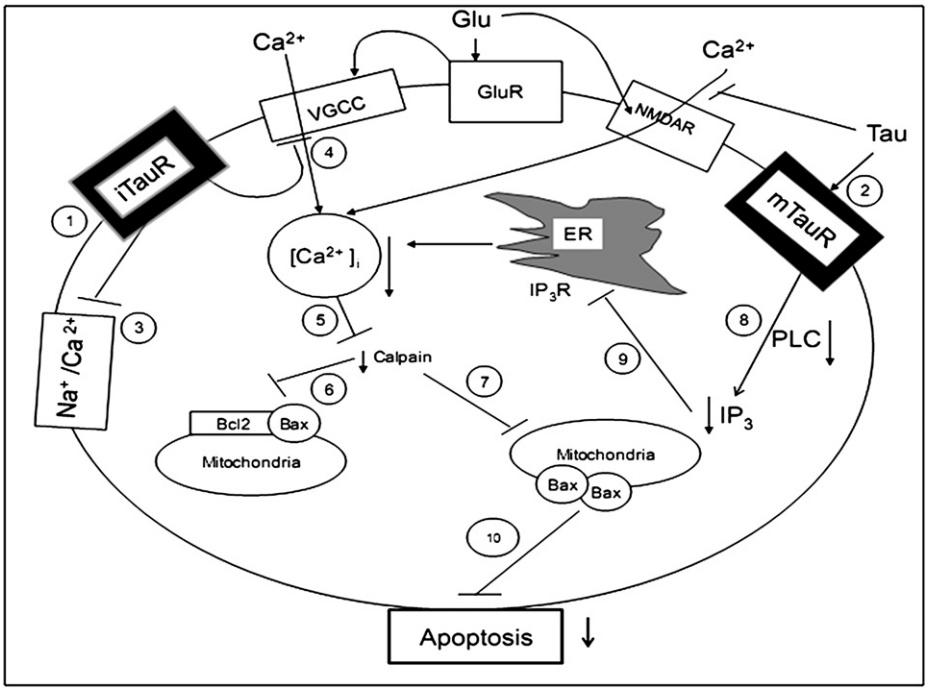

Figure 1 Summary schematic depicting the mode of action of taurine in neuroprotection. The sequence of events leading from the activation of taurine receptors to neuroprotection can be summarized as follows: (1) activation of ionotropic taurine receptors (iTauR) and/or activation of metabotropic taurine receptors (mTauR); (2) inhibition of the reverse mode of sodium/calcium exchangers; (3) inhibition of voltage-gated calcium channels (VGCC) by taurine-induced hyperpolarization; (4) inhibition of calpain resulting from the decrease in the intracellular free-calcium concentration; (5) inhibition of the cleavage of Bcl-2 and Bax by the inhibition of calpain; (6) inhibition of the formation of the Bax homodimer, leading to the inhibition of apoptosis; (7) activation of mTauR, which is negatively coupled to inhibitory G proteins, resulting in the inhibition of phospholipase C (PLC) activity and a decrease in IP3 production; (8) decreased IP3 level inhibits the release of calcium from the internal calcium storage pools, such as the ER, resulting in reduced ER stress and inhibition of apoptosis.

3. Mode of action of G-CSF in neuroprotection

G-CSF is a growth factor that is known to stimulate the proliferation and survival of hematopoietic cells.[83] G-CSF can penetrate the blood-brain barrier and plays a prominent role in the CNS.[35] G-CSF and its receptors are expressed in neurons throughout the brain and their expression is induced by ischemia, which is suggestive of an autocrine protective signaling mechanism.[35] An increasing amount of evidence indicates that G-CSF is neuroprotective and neuroregenerative both *in vivo* and *in vitro*. For example, G-CSF protects against neurodegeneration in a number of neurological disease models, such as Parkinson's disease,[32,84,85] Huntington's disease,[37] and cerebral ischemia.[86] The neuroprotective functions of G-CSF are further discussed below.

3.1. Suppression of multiple apoptotic pathways

G-CSF has been consistently cited as an attenuator of apoptosis. G-CSF is known to reduce the number of apoptotic cells identified by cleaved caspase-3-immunoreactive neurons and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells in neonatal hypoxic-ischemic rats.[87] G-CSF also extends its protective effects by downregulating a number of anti-apoptotic factors such as Bax, caspase-3, upregulated Bcl-2, Bcl-xL, and Pim-1, thereby synergistically preventing the release of cytochrome into the cytosol and translocating Bax to the mitochondria,[88–91] as shown in Figure 2.

In addition, G-CSF does not only modulate the intrinsic apoptosis pathway, but also the extrinsic apoptosis pathway by mediating its anti-apoptotic role through the tumor necrosis factor-related, apoptosis-inducing ligand (TRAIL) pathway.[33] Recombinant G-CSF reduces the number of TRAIL-positive neurons and protects senescence-accelerated mice against memory impairment.[33] Such properties make G-CSF an attractive therapy for the treatment of diseases characterized by dementia. In retinal ganglion cells, the anti-apoptotic properties of G-CSF have been attributed to the Phosphatidyl inositol 3-kinase (PI3)/AKT pathway and 6-hydroxydopamine (6-OHDA)-induced toxicity via the ERK pathway.[92,93] Recently, we reported that G-CSF alone, or in combination with taurine, protects glutamate-induced primary rat neuronal cultures by downregulating the ER stress markers GRP78, CHOP, Bim, and caspase-12 *in vitro*.[80] In addition to its neuroprotective functions, G-CSF also exerts effects on the neuroregenerative/stem cell mechanisms, as discussed in the following section.

3.2. Activation of cell proliferation mechanisms that promote neurogenesis

G-CSF stimulates the neural progenitor response *in vivo* and markedly improves long-term behavioral outcomes after cortical ischemia.[35] Peripheral infusion of G-CSF enhances the recruitment of progenitor cells from the lateral ventricle wall into ischemic areas of the neocortex in rats.[35] Furthermore, G-CSF is known to induce neurogenesis by activating signal transducer and activator of transcription 3 (STAT3), signal transducer and activator of transcription 5 (STAT5), and vascular endothelial growth factor (VEGF).[87,94,95] In an *in vivo* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson's disease, G-CSF significantly increases the number of dopamine (DA) neurons and the functions of the DA system, suggesting that G-CSF restores the degenerated nervous system through both neuroprotective and neurogenetic mechanisms.[84]

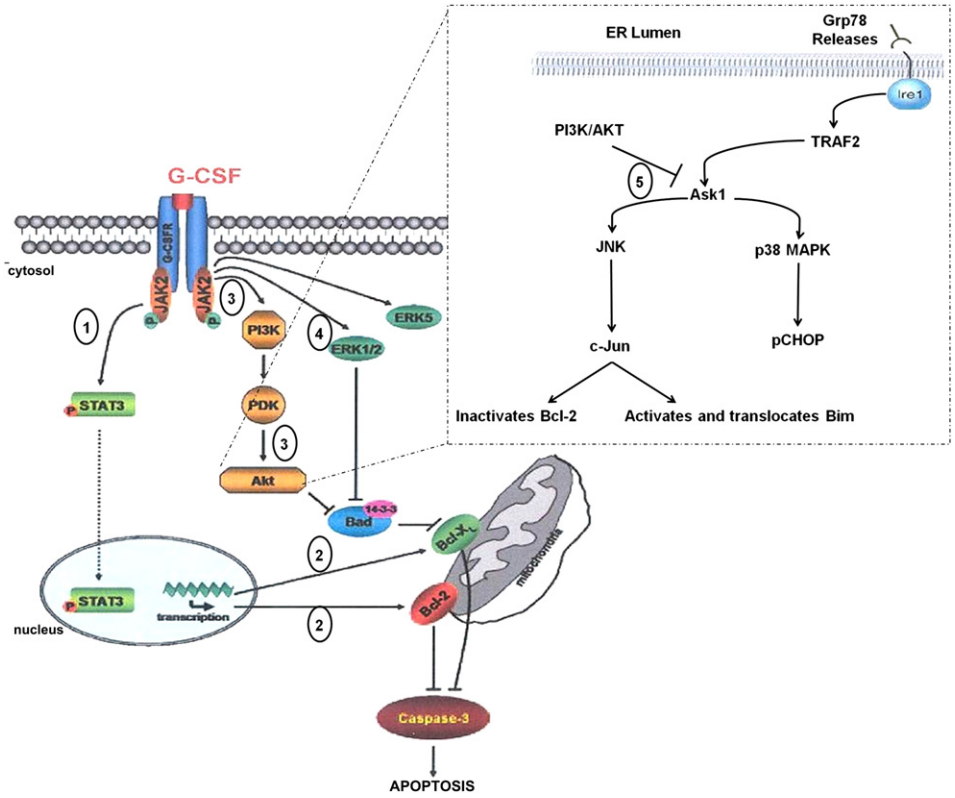

Figure 2 Proposed mode of action of the neuroprotective functions of G-CSF. G-CSF could exert its neuroprotective functions through one or more of the following signaling pathways: (1) activation of the STAT3 pathway results in translocation of STAT3 to the nucleus and (2) the upregulation of the anti-apoptotic genes, *Bcl-2* and *Bcl-X*; (3) activation of the PI3 K/AKT pathways or (4) ERK1/2 pathway results in the inhibition of the pro-apoptotic protein, Bad; (5) activation of the PI3 K/AKT pathways inhibits the ER stress-mediated ASK-1 pathway, resulting in disinhibition or activation of the anti-apoptotic protein, Bcl-2, and inactivation of the pro-apoptotic protein, BIM (see insert).

3.3. Clinical applications of G-CSF

In our recent studies, we have observed a remarkable improvement in neuronal functions, both in clinical cases and Parkinson's disease animal models that have been treated with G-CSF. Among the clinical cases, a patient with end-stage corticobasal ganglionic degeneration showed marked improvement after G-CSF treatment based on the patient's evaluation according to the Unified Parkinson's Disease Rating System (UPDRS). Overall improvement was 66% across all four categories: 1) mentation, behavior, and mood; 2) daily life activities; 3) motor skills; and 4) complications.[85] These results suggest that G-CSF may promote the regeneration of DA neurons in the substantia nigra and their functional integration into the nigrostriatal pathway. To extend this work, we conducted laboratory tests using the MPTP mouse model of Parkinson's disease. Unlike other published studies where G-CSF was administered before MPTP treatment,[32] in our study we opted for delayed treatment with G-CSF until after degeneration of the DA neurons by MPTP had been completed. We found that MPTP causes a marked loss in DA neurons, and G-CSF treatment restores the functions of the DA system, as indicated by increases in the number of DA neurons, stimulation-induced DA release, restoration of the nigrostriatal pathway, and improvement in locomotor activities, all of which are suggestive that the observed restoration might be due to differentiation of substantial nigra neuronal progenitor cells or progenitor cells that invade the substantia nigra after G-CSF application.[84,85] In addition to PD, we also found that G-CSF markedly reduces the size of brain infarctions that are induced by middle cerebral artery occlusion stroke animal model. These findings support the notion that G-CSF is a novel agent with both neuroregenerative/stem cell and neuroprotective activities and could be effective for the treatment of Parkinson's disease, stroke, and other degenerative brain disorders.

4. Conclusion

In the present review, we demonstrate that both taurine and G-CSF are attractive therapeutic targets for the treatment of neurodegenerative diseases. Both taurine and G-CSF function by suppressing apoptosis at multiple levels. The synergistic action of the combination of taurine and G-CSF has been proven to be beneficial for treating glutamate-induced neurotoxicity in primary rat neuronal cultures.[80] Because taurine is a natural amino acid and G-CSF is approved by the Food and Drug Administration, these findings could push forward the development of combinational approaches that provide more effective therapies.

Acknowledgments

This work was supported, in part, by the James and Esther King Biomedical Research Program, Florida Department of Health (grant #: 09KW-11), and the Schmidt Foundation, Charles E. Schmidt College of Medicine, Florida Atlantic University.

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