Filgrastim (G-CSF) ameliorates Parkinsonism l-dopa therapy’s drawbacks in mice

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**ABSTRACT**

L-dopa is still the cornerstone symptomatic medication for Parkinson disease (PD), although it cannot stop the neurodegenerative process progression or even aggravate it. Filgrastim (G-CSF) is a hematopoietic growth factor, exhibited neurotrophic, antioxidant, anti-apoptotic, immunomodulating and neuroprotective potentialities. The present study assessed the possible modulating potentialities of filgrastim on l-dopa treatment’s drawbacks in a mouse model of PD. Male BALB/c mice received 30 mg/kg/day rotenone suspended in 0.25 ml 0.5% CMC in PBS for 28 days orally from day 1st until the day 28th of the experiment for induction of PD. Since day 29th till day 43rd, mice treated with either 10 mg/kg/day l-dopa and 2.5 mg/kg/day carbidopa suspended in 0.25 ml 0.5% CMC in PBS orally, 50 μg/kg/day filgrastim in 0.1 ml 5% dextrose SC or a combination of both. Filgrastim, in the present study, able to alleviate the l-dopa therapy’s drawbacks in PD that revealed by the restoration of the exhausted nigrostriatal GSH level, and the reduction of the elevated nigrostriatal MDA, NO and TNF-α levels that deteriorated by l-dopa therapy. Moreover, the co-therapy of filgrastim with l-dopa, considerably potentiated the deteriorated mice’s working memory, and abrogated the nigrostriatal histopathological changes and caspase-3 immunohistochemical expression, failed to improve by l-dopa therapy. Furthermore, the filgrastim co-therapy with l-dopa demonstrated a remarkable improvement in the nigrostriatal dopamine level, and repression of rotenone-induced descent latency prolongation, as well as, stride length reduction than each alone. Therefore, filgrastim is promising, as a disease-modifying therapy, in amelioration of l-dopa therapy’s drawbacks in PD.

1. Introduction

Parkinson’s disease (PD) is a worldwide neurodegenerative disorder affecting approximately 1% of the sixty years old individuals all over the world, and its prevalence dramatically rises with age progress [1]. It’s characterized by a severe and progressive degeneration of nigrostriatal dopaminergic neurons, which manifested by motor symptoms such as bradykinesia, rigidity, resting tremors and gait disturbances, in addition to non-motor symptoms like cognitive impairment, behavioral disturbances, and autonomic nervous system impairment [2,3]. Free radical generation, mitochondrial dysfunction, neuroinflammation, apoptosis, excitotoxicity, and trophic factor deficiency incriminated in its development. Moreover, complex interactions of multiple environmental and genetic factors are required for its induction [4–9].

Many strategies developed for PD treatment; however, l-dopa is still the cornerstone symptomatic PD medication, although it cannot stop the underlying neurodegenerative process progression [10]. Furthermore, it proved neurotoxic to nigrostriatal dopaminergic neurons, through free radical generation, apoptosis induction, and augmentation of neuroinflammation, resulting in resistance to its therapeutic effects, with the subsequent fluctuation of its motor and non-motor profits, and further progression of PD [11–13]. Moreover, it has many side effects, especially psychosis, aberrant sexual behavior, and dyskinesia, which encountered in nearly 40% of cases after 4–6 years and increasing to be 90% by 10 years of l-dopa treatment [14,15]. Therefore, the search for a medication that prevents l-dopa treatment’s adverse effects, and modulates the progressive course of the disease is mandatory.

Granulocyte-colony stimulating factor (G-CSF), filgrastim, is one of the hematopoietic growth factors used for the management of neutropenia [16]. Its advantage over the other hematopoietic growth factors is its ability to cross the blood-brain barrier [17]. Recently, it exhibited neurotrophic, antioxidant, anti-apoptotic, immunomodulating, and neuroprotective potentialities [18–24]. In addition, it improved the...
outcome of many neurological disorders such as cerebral ischemia, spinal cord injury, and Alzheimer’s disease [21,23,24]. Furthermore, it exhibited neuroprotective potentialities for multiple neurodegenerative disorders such as Huntington’s disease, amyotrophic lateral sclerosis and PD [25,26]. Meanwhile, although it administered by more than 3 million patients, it exhibited minimal adverse effects [27].

Therefore, in the present study, we investigated the possible modulating potentialities of filgrastim on l-dopa treatment’s drawbacks, through evaluation of motor behavioral outcomes, working memory, nigrostriatal dopaminergic level, oxidative stress markers, tumor necrotizing factor-alpha (TNF-α) level, histopathological changes and immunohistochemical caspase-3 expression in a mouse model of PD.

2. Material and methods

2.1. Drugs & reagents

The following drugs and reagents obtained commercially and used, rotenone and diaminobenzidine (Sigma, St., Louis, MO, USA), carboxymethyl cellulose (CMC), phosphate-buffered saline (PBS), Tris-HCl buffer, formalin buffered saline, hydrogen peroxide, methanol, citrate buffer, hematoxylin and eosin stains (El Gomhuria Co., Tanta, El-Gharbeya, Egypt), l-dopa and carbidopa (Global Nabi Co., Ramses, Cairo, Egypt), filgrastim (Roche Co., Basel, Switzerland), saline 0.9% and dextrose 5% solution (Otsuka Egypt Co., Nasr city, Cairo, Egypt).

2.2. Animals

The study carried out using sixty, eight-weeks-old male BALB/c mice, with an average weight 20–25 g that obtained from Tanta University Animal House. All animals housed in wire mesh cages at 20 ± 2 °C, with a relative humidity of 65 ± 10%, exposed to a 12-hour light/dark cycle, fed a standard laboratory diet and water ad libitum and allowed to adapt for one week before starting the experiments. All experiments carried out following the guidelines for the care and use of experimental animals in Faculty of Medicine, Tanta University, Egypt, with an approval of the Tanta Faculty of Medicine’s Animal Experiment Ethics Committee.

2.3. Experimental design

Mice randomly divided into 6 groups of 10 mice each. Group 1 (CONT), normal mice served as a control group and received 0.25 ml/day 0.5% CMC in PBS orally for 28 days from the 1st day until the 28th day of the experiment. Group 2 (ROT), PD induced mice by receiving 30 mg/kg/day rotenone suspended in 0.25 ml 0.5% CMC in PBS for 28 days orally from the 1st day till the 28th day of the experiment [28]. Group 3 (VEH), PD induced mice treated with 0.25 ml 0.5% CMC orally and 0.1 ml 5% dextrose subcutaneously (SC) for 15 days from the 29th day till the 43rd day of the experiment. Group 4 (LD), PD induced mice treated with 10 mg/kg/day l-dopa and 2.5 mg/kg/day carbidopa (peripheral dopa decarboxylase inhibitor) suspended in 0.25 ml 0.5% CMC in PBS for 15 days orally from the 29th day till the 43rd day of the experiment. Group 5 (FIL), PD induced mice treated with 50 μg/kg/day filgrastim in 0.1 ml 5% dextrose SC for 15 days from the 29th day till the 43rd day of the experiment [22]. Group 6 (LF), PD induced mice treated with a combination of 10 mg/kg/day l-dopa and 2.5 mg/kg/day carbidopa suspended in 0.25 ml 0.5% CMC in PBS orally, and 50 μg/kg/day filgrastim in 0.1 ml 5% dextrose SC for 15 days from the 29th day until the 43rd day of the experiment (Fig. 1). All suspensions prepared fresh daily, and all animals fasted for 2 h daily before administration of treatment, which introduced from 9:00 until 11:00 A.M.

2.4. Data and sample collection

Four hours after the last treatment, all mice’s behavioral tests evaluated. Then, twenty-four hours after the last treatment, mice sacrificed by cervical dislocation, mice’s brains immediately dissected, washed with ice-cold saline, and the basal ganglia of the two hemispheres harvested. The left basal ganglia immediately immersed in 10% formalin buffered saline and used for evaluation of histopathological changes and immunohistochemical caspase-3 expression. Meanwhile, the right basal ganglia homogenized in Tris-HCl buffer to give 10% homogenate. Half of the homogenate centrifuged at 5000 rpm for 10 min and the supernatants collected. The homogenate and its supernatant, stored at −80°C for further estimation of neurochemical parameters.

2.5. Behavioral tests evaluation

2.5.1. Catalepsy test

Catalepsy test performed to quantify the motor manifestations of Parkinsonism as described by Alam and Schmidt. In brief, it performed in two steps, the first step, the grid test, where each mouse hung by all four paws on a vertical grid, which measured 25.5 cm width and 44 cm height with a space of 1 cm between each grid, then a stopwatch started as soon as the mouse held onto the grid. Just as the mouse moved its paw or showed the first movement, the stopwatch stopped and the time noted as descent latency. Then, the second step of the test, the bar test, was done. Briefly, the mouse placed with both front paws on a bar, which was 9 cm above and parallel to the base, in a half rearing position, and the time counted by stopwatch. When the mouse removed one paw from the bar, the stopwatch stopped, and the time noted. The maximum descent latency for both the grid and the bar test fixed at 180 s [29].

2.5.2. Stride length test

Stride length test achieved to analyze the mice’s gait as noted by Fernagut et al. (2002). Shortly, the animals trained three times to walk through a narrow alley leading into their home cage. Then, a white paper on a narrow wooden plank placed on the alley floor, each mouse’s forelimbs and hindlimbs brushed with commercial nontoxic watermark ink and placed at the beginning of the alley. As it walked to their home cage, they left their paw prints on the paper underneath, and the distance between two paw prints measured manually as stride lengths. The longest stride length considered as the maximal velocity for each run, and then the average between the forepaw and hindpaw stride lengths calculated. Runs, where the mice made stops or obvious decelerations observed and excluded [30].

2.5.3. T-maze spontaneous alternation test

T-maze spontaneous alternation test accomplished to measure mice’s working memory, according to Deacon and Rawlins. In brief, each mouse moved to the examination room for 10 min, then placed in the start zone of the T-maze apparatus, which is a black painted wooden T form apparatus placed horizontally measuring 30 cm length, 10 cm width, and 20 cm height, for start alley and the goal arms. The mouse allowed to choose the goal arm, where it confined in for 30 seconds, then moved to its cage for 1 min. Afterward, it took again to the start arm to begin the second trial. This trial repeated for five consecutive times. Alternation was recorded when the mice accessed one arm in the first trial and chose the other arm in the second trial. The percentage of alternation for each animal calculated [31].

2.6. Neurochemical parameter estimation

2.6.1. Estimation of nigrostriatal protein level

Nigrostriatal protein level measured with a colorimetric assay kit obtained from Bio-Diagnostic Co., Dokki, Giza, Egypt, according to the method designated by Fleury and Eberhard (1950). Briefly, 0.025 ml of tissue homogenate added to 1 ml of Biuret reagent, mixed well and incubated for 10 min at 37°C. Nigrostriatal protein level determined by
measuring the optical densities at 550 nm using Biosystems semiautomatic analyzer (BTS-350, Barcelona, Spain), and expressed as mg/ml tissue homogenate [32].

2.6.2. Estimation of nigrostriatal dopamine level

The nigrostriatal dopamine level assessed according to the method of Kim et al. using a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) kit, obtained from Glory Science Company, Del Rio, USA, with a minimum detection limit of 0.005 ng/ml. The optical densities estimated with an automated plate reader (Stat Fax 2100, Fisher Bioblock Scientific, BP., Illkirch Cedex, France), and the nigrostriatal dopamine levels expressed as ng/mg tissue protein [33].

2.6.3. Estimation of nigrostriatal TNF-α level

The nigrostriatal TNF-α level assessed using DAS-ELISA kit obtained from Sunred Biotechnology Co., Shanghai, China, with a minimum detection limit of 0.002 ng/ml. The tissues homogenate TNF-α levels measured according to the method of Beutler et al. with an automated plate reader (Stat Fax 2100, Fisher Bioblock Scientific, BP., Illkirch Cedex, France), and the nigrostriatal TNF-α expressed as ng/mg tissue protein [34].

2.6.4. Estimation of nigrostriatal oxidative stress marker levels

Nigrostriatal malondialdehyde (MDA), reduced glutathione (GSH) and nitrite levels were estimated to determine the tissue oxidative stress state, using colorimetric assay kits obtained from Bio-Diagnostic Co., Dokki, Giza, Egypt. MDA levels evaluated from Sunred Biotechnology Co., Del Rio, USA, with a minimum detection limit of 0.002 mg/ml. The tissue homogenate MDA levels measured according to the method nominated by Kei. Concisely, 0.2 ml of tissue homogenate added to 1 ml of thiorbarbituric acid, heated in boiling water bath for 30 min, allowed to cool down, and the optical densities measured at 534 nm [35]. GSH level was assessed according to Beutler et al. Briefly, 0.5 ml of tissue homogenate added to 0.5 ml trichloroacetic acid, mixed well, allowed standing at room temperature for 5 min, centrifuged at 3000 rpm for 15 min and the supernatant harvested. Then 0.5 ml of the supernatant mixed well with 1 ml of 0.3 M sodium phosphate buffer solution and 0.1 ml of 5, 5’ dithio 2-bisnitrobenzoic acid, and the optical densities analyzed at 405 nm [36]. The levels of nitrites (the final products of NO metabolism) determined, basing on the method of Montgomery and Dymock. Shortly, 0.1 ml of tissue homogenate mixed well with 1 ml of 1% sulfanilamide, allowed to stand at room temperature for 5 min, then 0.1 ml of 0.1% N-1-naphthyl ethylenediamine dihydrochloride added and mixed well. After 5 min of standing at room temperature, the optical densities measured to assess the nitrites levels at 540 nm [37]. All optical densities evaluated using Biosystems semiautomatic analyzer (BTS-350, Barcelona, Spain), and the levels of MDA, GSH and nitrites expressed as nmol/mg tissue protein.

2.7. Evaluation of nigrostriatal histopathological changes

2.7.1. Hematoxylin and eosin (H & E) stain

According to Suvarna and Layton (2013), 5 μm sections of basal ganglia tissues stained with H & E stain, mounted with Canada balsam, examined under a light microscope (Olympus CX21, Japan) and evaluated for neuron shape (shrinkage), neuropil vacuolation, Lewy-like pathology (LBs) and multinucleated giant cells [38]. The nigrostriatal H & E changes scored arbitrarily as follow; 0, no, 1, mild, 2, moderate and 3, sever changes.

2.7.2. Immunohistochemical expression of caspase-3

The basal ganglia’s caspase-3 immunohistochemical expression evaluated according to the method described by Buchwalow and Böcker (2010). In brief, basal ganglia tissue’s sections deparaffinized, rehydrated, incubated with 0.3% hydrogen peroxide/methanol for 20 min to block the endogenous hydrogen peroxidase activity. Afterward, it washed with PBS for 3 times, placed in 10 mM citrate buffer solution (pH 6.0) and then retained in serum-free protein-blocking solution (Block Ace, DS Pharma Biomedical Co., Ltd., Osaka, Japan) for 20 min at room temperature to block the nonspecific protein binding sites. Next, it incubated overnight at 4 °C with anti-caspase-3 polyclonal antibody (1:100 in PBS, Lab Vision Inc., Altrincham, Cheshire, UK), washed with PBS for 3 times, incubated for 10 min at room temperature with secondary biotinylated goat anti-polyvalent antibodies (Lab Vision Inc., Altrincham, Cheshire, UK), then 2 drops of diaminobenzidine added as a chromogen. Subsequently, sections counterstained with Mayer’s hematoxylin, dehydrated, cleared, mounted and examined under a light microscope (Olympus CX21, Japan), where the positive reaction appeared brown, while the negative one was blue [39]. The percentage of the total cell’s positive reaction scored according to Bodey et al. (2004) as follows 0, 1% are positive, 1, 2–10% are positive.
2. 11–50% are positive, 3, 51–90% are positive and 4, over 90% are positive [40].

2.8. Statistical analysis

The experimental data's statistical differences evaluated using IBM Statistical Package for the Social Sciences (SPSS) Software for Windows (version 23, IBM Corp., Armonk, NY, USA). The variances of data analyzed with either one-way ANOVA (followed by Tukey’s test as a post hoc test) or Kruskal-Wallis’s test (followed by Mann-Whitney’s U-test as a post hoc test), after analysis of variances with Bartlett’s test. The data of the present study expressed as mean ± SD and the significance considered when P values < 0.05.

3. Results

3.1. Effect of filgrastim on behavioral outcomes

3.1.1. Motor manifestation

The repeated rotenone administration significantly prolonged the descent latency in both grid and bar test, as well as, it reduced the stride length. These deviations effectively abrogated with l-dopa and filgrastim treatment each alone and in combination. However, l-dopa treatment was superior to filgrastim treatment in amelioration of these motor deviations. Furthermore, the combination regimen ameliorates these deviations efficiently than the treatment with l-dopa and filgrastim each alone (Fig. 2).

3.1.2. Working memory

Filgrastim treatment considerably enhanced the mice’s working memory, presented by percentage of spontaneous alternation improvement, which deteriorated by the repeated rotenone administration. Moreover, l-dopa and filgrastim combination therapy showed an upper hand than filgrastim therapy in the improvement of the mice’s working memory. In contrary, l-dopa therapy failed to show any significant effect on the memory function (Fig. 2).

3.2. Effect of filgrastim on nigrostriatal dopamine level

The nigrostriatal dopamine level severely exhausted, by the repeated rotenone administration. This exhaustion significantly alleviated by l-dopa and filgrastim treatment each alone and in combination, with an obvious superiority to combination regimen over treatment with each alone. Moreover, l-dopa treatment effectively restored the nigrostriatal dopamine level than filgrastim treatment alone (Fig. 3).

3.3. Effect of filgrastim on nigrostriatal TNF-α level

Filgrastim therapy and combination regimen significantly suppressed the elevated TNF-α level, which elevated with repeated rotenone administration, with no significant difference in between. However, l-dopa therapy augmented the elevated TNF-α level, which raised with repeated rotenone administration, when compared with rotenone group (Fig. 3).

Fig. 2. Effect of filgrastim on behavioral outcomes of rotenone-induced PD in male BALB/c mice. The results expressed as mean ± SD of 10 mice per each group. CONT: Normal mice served as a control group. ROT: mice receiving 30 mg/kg/day rotenone suspended in 0.25 ml 0.5% CMC in PBS for 28 days orally for induction of PD. VEH: PD induced mice treated with 0.25 ml 0.5% CMC orally and 0.1 ml 5% dextrose SC for 15 days. LD: PD induced mice treated with 10 mg/kg/day l-dopa and 2.5 mg/kg/day carbidopa suspended in 0.25 ml 0.5% CMC in PBS for 15 days orally. FIL: PD induced mice treated with 50 μg/kg/day filgrastim in 0.1 ml 5% dextrose SC for 15 days. LF: PD induced mice treated with a combination of both l-dopa and filgrastim for 15 days. * P < 0.05, ** P < 0.01 and *** P < 0.001 (vs. ROT group), + P < 0.05, ++ P < 0.01 and +++ P < 0.001 (vs. LD group), and & P < 0.05, && P < 0.01 (vs. FIL group).
3.4. Effect of filgrastim on nigrostriatal oxidative stress markers

Parallel with nigrostriatal TNF-α level, filgrastim therapy, and combination regimen significantly enhanced the antioxidant state of the nigrostriatal tissues, which indicated by remarkable repression of the elevated nigrostriatal MDA and NO levels, meanwhile, the elevation of the decreased GSH level, with no significant difference in between. In contrary, l-dopa aggravated the exhaustion of the nigrostriatal antioxidant state that revealed by further elevation of nigrostriatal MDA and NO levels and reduction of GSH level than the rotenone group (Fig. 4).
3.5. Effect of filgrastim on basal ganglia histopathological changes of H&E stained sections

Rotenone and VEH groups showed shrunken, irregular pyramidal and granular cells, leaving pericellular empty spaces, along with intracytoplasmic LBs, neuropil cytoplasmic vacuoles as well as large multinucleated giant cells. These changes were in comparison with the normal histological structure of the basal ganglia, where the pyramidal and granular cells haphazardly distributed, with pale nuclei, prominent nucleoli, and basophilic cytoplasm, in addition to blood capillaries, neuroglial cells, and neuropil in between the nerve cells. Filgrastim treatment and the combination regimen significantly abrogated the histopathological changes induced by repeated rotenone administration, with no significant difference in-between (Figs. 5 & 7).

3.6. Effect of filgrastim on basal ganglia immunohistochemical caspase-3 expression

Rotenone, VEH, and LD groups showed a strong positive caspase-3 reaction in comparison to the negative reaction of the control group. However, the combinations of filgrastim with l-dopa and filgrastim therapy alone efficiently ameliorated immunohistochemical caspase-3 expression, which induced by repeated rotenone administration, with no significant difference in-between (Figs. 6 & 7).

4. Discussion & conclusions

Although many therapeutic strategies developed for the treatment of PD, l-dopa remains the gold standard therapeutic agent in the improvement of the motor symptoms [10]. Nevertheless, it cannot stop the progressive neurodegenerative course of the disease, but it also aggravates that course through multiple neurotoxic mechanisms, thus, l-dopa therapy could contribute to the bad prognosis of PD [11–13].

The findings of the present study elucidated the ability of filgrastim to ameliorate the drawbacks of l-dopa therapy, which evidenced by the restoration of the exhausted nigrostriatal antioxidant activity and the reduction of the elevated nigrostriatal pro-inflammatory cytokines, which induced by the repeated rotenone administration and deteriorated by l-dopa therapy. These findings indicated by significant enhancement of the nigrostriatal GSH level and repression of nigrostriatal MDA, NO and TNF-α level, with filgrastim and l-dopa combination therapy. Moreover, the co-therapy of filgrastim with L-dopa, considerably potentiated the deteriorated mice’s working memory, and abrogated the nigrostriatal histopathological changes and caspase-3 immunohistochemical expression, failed to improve by l-dopa therapy. Furthermore, the filgrastim co-therapy with l-dopa demonstrated a remarkable improvement in the nigrostriatal dopamine level, and repression of rotenone-induced descent latency prolongation, as well as,
stride length reduction than each alone. Additionally, filgrastim treatment alone showed similar results to the combination regimen, except for the behavioral parameters and the nigrostriatal dopamine level, where the combination therapy was superior.

The deterioration of nigrostriatal antioxidant activity, with overexpressed ROS and RNS, is one of the pathognomonic mechanisms of PD development and progression, particularly as the turnover rate of nigrostriatal membrane phospholipid is low, and thus rendering them unable to respond to oxidative stress, with subsequent apoptosis and neuronal damage [5,41,42]. Treatment of PD with L-dopa, which transformed to dopamine in nigrostriatal cells, control the motor manifestations of PD, but at the same time generates more ROS and RNS, as demonstrated in the present study, with further destruction of functioning cells. These generated ROS and RNS are not only due to L-dopa metabolism, but also directly by l-dopa and dopamine itself, since L-dopa treatment increase cytosolic dopamine content, as L-dopa

Fig. 6. A, -ve control: Negative caspase-3 reaction. B, CONT group showing neurons with –ve caspase-3 (→) however, few cells showing light brown cytoplasmic granules (►). C, ROT group showing a strong positive caspase-3 reaction of the neurons (→). D, VEH group showing a nearly similar reaction to rotenone group (→). E, LD group showing an apparently aggravated caspase-3 reaction. F, FIL group showing a nearly normal picture. G, FL group showing some neurons expressed positive reactions for caspase-3 (→) (Caspase-3 × 400).
and dopamine are auto-oxidized to highly reactive compounds including quinones [10,43]. In addition, L-dopa rapidly oxidized in extracellular space by non-enzymatic process accelerated with iron to form highly reactive compounds, ROS and RNS [44]. Furthermore, Long-term treatment with L-dopa inhibits ubiquitin-proteasome system, responsible for degradation of misfolded dysfunctional proteins like α-synuclein, thus accumulation of such proteins that can interact with L-dopa, dopamine and its oxidative products with additional formation of ROS and RNS, hence, further mitochondrial damage, energy store failure and apoptotic pathway induction [45,46].

Data of the present study showed that either the filgrastim therapy alone or as a co-treatment with L-dopa effectively ameliorated the exhausted nigrostriatal antioxidant activity. These data are in accordance with Park et al. who reported that filgrastim are able to enhance antioxidant enzyme activity and abrogate lipid peroxidation in a rat model of Alzheimer’s disease, through enhancement of neuronal progenitor cell proliferation [13]. In the same context, Hou et al. documented the capability of filgrastim to protect cardiac tissues, through inhibition of tissue peroxidative alterations, which manifested by a significant reduction of MDA and augmentation of GSH levels [47]. Moreover, Deetjen et al. and Komine-Kobayashi et al. reported that filgrastim treatment suppresses the inducible nitric oxide synthase (iNOS) gene expression and protein synthesis, thus reducing NO release in vascular smooth muscle cells in vitro, and transient focal ischemia, respectively [48,49]. Additionally, Song et al. showed that peg-filgrastim are capable of suppressing dopamine turnover, and hence decreasing its oxidative metabolites and cellular stress in a mice model of PD [17].

Although, the low levels of pro-inflammatory cytokines, such as TNF-α and interleukin (IL)-1β, promote the production of neurotrophic factors, however, there is increasing evidence that its higher levels in PD activate inflammatory and apoptotic pathways, which involved in nigrostriatal neurodegeneration [50]. Data of the present study revealed that L-dopa treatment increased the pro-inflammatory cytokine, TNF-α, which believed to be due to activation of microglia [51]. The raised pro-inflammatory cytokines enhance the inflammatory process progression as well as encouraging iNOS, myeloperoxidase and NADPH oxidase expression, with subsequent ROS and RNS generation [52]. Moreover, TNF-α is cytotoxic to dopaminergic neurons, either through stimulation of TNF-α receptor type 1, which enhance the apoptotic cascades or by activation of iNOS, with consequent augmentation pro-inflammatory cytokine production, thus amplification of the inflammatory response [6,10]. Additionally, TNF-α phosphorylates and degrades the inhibitory factor IkB resulting in activation of nuclear factor-κB (NF-κB) signaling cascade, with the subsequent promotion of iNOS production from activated microglia thus augment production of NO, which in turn has a detrimental effect on dopaminergic neurons proteins and DNA [53].

Impressively, filgrastim alone or in combination with L-dopa the reduced the raised nigrostriatal TNF-α level, with the alleviation of the augmented nigrostriatal TNF-α level induced by L-dopa therapy. Filgrastim exert these effects through modulating the response of microglia to stress, thus prevent its activation, with subsequent suppression of pro-inflammatory cytokines production, and alleviation of the inflammatory response, as well as, the promotion of neurotrophic cytokine expression [18,21]. Moreover, it inhibits iNOS activity and NF-κB signaling, resulting in suppression of NO production and remission of the inflammatory response [21,49].

In fact, there is a positive correlation between the degree of PD patients’ dopaminergic neurons loss, and the percentage of nigrostriatal caspase-3 expression [54]. Interestingly, our present data established that the filgrastim therapy either alone or as a co-treatment with L-dopa, effectively suppressed the immunohistochemical caspase-3 expression, induced by rotenone. Which, could be explained by the ability of filgrastim to stimulate phosphatidylinositol 3-kinase/protein kinase B, extracellular-signal-regulated kinase and Signal transducer and activator of transcription-3 (STAT-3) pathways. That, in turn, inhibits p53 upregulated modulator of apoptosis and Bax that considered as cell death-inducing proteins, meanwhile, it induces the anti-apoptotic proteins, Bcl-2, expression and thus inhibits caspase-3 expression [23,55,56]. Moreover, filgrastim directly stimulates the G-CSF receptors on nigrostriatal neurons and neural progenitor cells, leading to enhancement of its plasticity and neurogenesis, and prevention of its degeneration and apoptosis [57]. In addition, the activation of G-CSF receptors by filgrastim stimulate the STAT-3 pathway with subsequent suppression of ROS and RNS generation, and inhibition of caspase-3 activity [19]. Furthermore, filgrastim inhibits neuroinflammation; by suppressing pro-inflammatory cytokines production and iNOS activity, therefore, repress apoptosis [21].

Cognitive function impairment is one of the PD abhorrent sequelae, with many mechanisms proposed for its development, including dopaminergic neurons loss, mitochondrial dysfunction, exhaustion of antioxidant activity, neuroinflammation and reduction of neurotrophic factors levels [4–9]. The treatment with L-dopa is of no benefit in the treatment of cognitive function impairment in PD or in stopping of its progression, however, it may even aggravate it, through ROS and RNS generation, induction of microglial activation, neuroinflammation,
apoptosis, and serotonin metabolism disorder [25,58,59]. Notably, the treatment of PD with filgrastim alone or in combination with L-dopa, in the present study, improved the mice’s working memory greatly, indicating the improvement of mice’s cognitive function, which did not affect with L-dopa treatment alone. Moreover, the filgrastim and L-dopa combined therapy ameliorated the PD’s motor activity deviations effectively than L-dopa and filgrastim therapies each alone. From the data of the present study, the beneficial effects of filgrastim co-therapy could be mediated through enhancement of neuron antioxidant activity, suppression of neuroinflammation, and inhibition of neuronal apoptosis. Matching with our data, Zhao et al. reported that administration of filgrastim significantly protected against spatial memory impairment, through inhibition of hippocampal neurons apoptosis in a mouse model of senile dementia [60]. Moreover, Liao et al. demonstrated that filgrastim enhanced the rat’s working memory function in a chronic cerebral ischemia by suppression of hippocampal astrocyte apoptosis, and by promoting its proliferation [22]. However, Prakash et al. attributed the improvement in the rotational behavior of a severe PD model after filgrastim administration, to the enhancement of striatal presynaptic axonal sprouting rather than the increase dopamine neurons survival or prevention of its apoptosis [23]. Furthermore, Liew et al. certified that filgrastim enhanced the neurobehavioral profile in a rat model of Alzheimer’s disease through induction of neuro-cerebral progenitor cell proliferation [24]. In conclusion, filgrastim, in the present study able to alleviate the L-dopa therapy’s drawbacks in PD, through the improvement of the deteriorated nigrostriatal antioxidant activity and the reduction of the elevated nigrostriatal pro-inflammatory cytokines that augmented by L-dopa therapy. Moreover, it potentiated the deteriorated mice’s working memory, and abrogated the nigrostriatal histopathological changes and caspase-3 immunohistochemical expression, failed to improve by L-dopa therapy. Furthermore, it remarkably improved the nigrostriatal dopamine level, and behavioral parameter than filgrastim and L-dopa each alone. However, further investigations are needed to verify the possible beneficial effects of filgrastim on prolonged L-dopa therapy. Therefore, filgrastim could be promising, as a disease-modifying therapy, in amelioration of L-dopa therapy’s drawbacks in PD.

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