

Research Article



Effect of Tempol on Lipopolysaccharide Induced Amyloidogenesis, Neuroinflammation

Waleed Abd El-Salam Ibrahim Khallaf^{1*}, Basim Anwar Shehata Messiha¹, Amira Morad Hussein Abo-Youssef¹, Nesrine Salah El-Dine El-Sayed²

¹Department of Pharmacology and Toxicology, Faculty of Pharmacy, Beni-Suef University, Egypt.

²Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Egypt.

*Corresponding author's E-mail: waleedkhalaf@yahoo.com

Accepted on: 15-06-2015; Finalized on: 31-07-2015.

ABSTRACT

Alzheimer's disease (AD) is a progressive degenerative disorder which is accompanied with amyloidogenesis and oxidative stress. The possible mechanism by which the peripheral inflammatory response may affect the oxidative stress, neuroinflammation is via the expression of amyloid beta ($A\beta$). Tempol considered reactive oxygen species (ROS) scavenging agent having antioxidant and reduce amyloid β brain level. The aim of the present study aims to investigate the possible protective effects of tempol on LPS induced amyloidogenesis and neuroinflammation. Mice were divided into a normal control group, an Alzheimer control group receiving a single intraperitoneal (i.p) dose of LPS (0.8 mg/kg), a tempol (100 mg/kg/day, i.p.) receiving group. Biochemical tests which were performed included assessment of Amyloid β brain levels as an Alzheimer biomarker. Super oxide dismutase (SOD), reduced glutathione (GSH) and Malondialdehyde (MDA) as oxidative stress markers. Tempol corrected effect of excess amyloid β deposition which accompanied with neuronal degeneration and improved oxidative stress parameters as it increased GSH and SOD and decreased MDA levels leading to improved. Tempol considered promising drug improving neuroinflammation and amyloid β deposition induced by lipopolysaccharide and reduced oxidative stress which causes neuronal degeneration.

Keywords: Alzheimer's disease, Lipopolysaccharide, Tempol, Amyloid, Oxidative stress.

INTRODUCTION

Alzheimer's disease (AD) is a progressive degenerative disorder. The main neuropathological hallmarks of AD are the formation of senile plaques (SPs) following neurofibrillary tangles (NFTs) which causes neuronal degeneration and synaptic loss. In particular, the senile plaques are extracellular aggregates of the amyloid beta-peptide ($A\beta$) that is cleaved from the amyloid precursor protein (APP).¹

Neuropathological studies in the human brains have proved that the activation of glial cells excessively releases proinflammatory mediators and cytokines, oxidative stress which followed by triggering a neurodegenerative cascades via neuroinflammation.²

Several theories might explain pathogenesis of AD, including cholinergic hypothesis³⁻⁵, tau protein hypothesis^{6,7} and amyloid hypothesis.⁸

Deposition of beta amyloid ($A\beta$) plaques may activate the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, resulting in increased rate of ROS formation.⁹⁻¹¹

Amyloid- β proteins ($A\beta$) of 42 ($A\beta_{42}$) accumulate as senile plaques (SP) and cerebrovascular amyloid protein deposits that are defining diagnostic features of Alzheimer's disease (AD) so $A\beta$ accumulation is therefore a key pathological event and a prime target for the prevention and treatment of AD.¹²

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is a member of a family of nitroxide compounds that has

been studied extensively in animal models of increased reactive oxygen species and its effects on hypertension and endothelial function.¹³

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine- N-oxyl) is an antioxidant that works as a superoxide dismutase mimic, directly reacts with both carbon-centered and peroxy radicals, and prevents the reduction of hydrogen peroxide to the hydroxyl radical. Furthermore, this nitroxide can oxidize reduced transition metals that would otherwise serve to catalyze the formation of the hydroxyl radical via the Fenton reaction.¹⁴

Tempol was well-known as a ROS scavenger.¹⁵⁻¹⁷ It was selected as a reference treatment as oxidative stress was believed not only to be a result of $A\beta$ plaque deposition but also a causative factor, triggering brain $A\beta$ formation and deposition.^{18,19}

The aim of the present study aims to study the possible protective effects of tempol on lipopolysaccharide induced amyloidogenesis, neuroinflammation and oxidative stress.

MATERIALS AND METHODS

Animals

Adult male mice, 25±2 g, were purchased from the National Research Center, Giza, Egypt.

Animal handling and experimental procedures were carried out according to the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).



Drugs, Chemicals and Reagent kits

Tempol, LPS, thiobarbituric acid, 1,1,3,3-tetramethoxypropane, 5,5'-dithio-bis-(2-nitrobenzoic acid), and glutathione (GSH) standard were purchased from Sigma Chemicals Co. (USA).

ELISA kits of murine A β ₁₋₄₂ was obtained from MyBioSource Co. All other chemicals, solvents and reagents were of analytical grade.

Experimental Design

Animals were divided into three groups: a normal control group received saline intraperitoneal (i.p) injection, Alzheimer control group received lipopolysaccharide (0.8 mg/kg) for seven days and third group received tempol (100 mg/kg) plus LPS (0.8 mg/kg).

Twenty-four hours later, animals were sacrificed and brain tissue withdrawn for biochemical studies.

Methodology

Induction of amyloidogenesis, neuroinflammation

Amyloidogenesis, neuroinflammation induction was performed by a single i.p. dose of LPS (0.8 mg/kg), suspended in 1% tween 80 solution in saline.^{17,20} Tempol was administered once daily via the i.p. route for 7 days starting from the day of LPS administration.

Tissue preparation

Animals were sacrificed by decapitation with the brains removed rapidly in ice-cold saline twenty-four hours after the last dose of test agents or vehicles then they were homogenized in cold saline using a homogenizer (yellow line, DI18 basic, Germany), centrifuged at 4000 rpm at 4°C for 15 min using a cooling centrifuge (Sigma 3-30k, USA), and the supernatant was withdrawn and kept in a deep freezer at -80°C (Als Angelantoni Life Science, Italy) till the time of assay of A β , Super oxide dismutase (SOD), reduced glutathione (GSH) and Malondialdehyde (MDA).

Biochemical Tests

Alzheimer biomarker

Brain tissue levels of A β was measured based on the principles described by Verges.²¹

Oxidative stress markers

Brain tissue SOD was measured as described by Robak and Gryglewski.²² Brain tissue MDA and GSH levels were assessed according to the principles of Ohkawa²³ and, respectively and Ellman²⁴, respectively.

Data presentation and statistical analysis

Data were expressed as means \pm SEM (standard error of the mean). Statistical analysis was performed using statistical package for social sciences (SPSS) computer software (version 22). One-way analysis of variance (ANOVA) test was applied, followed by Tukey's post-hoc test to compare mean values pair-wise at $p < 0.05$.

RESULTS

Biochemical Estimation

Alzheimer marker

Group received LPS (0.8 mg/kg) showed significant increase of A β content when compared with normal control group, while group received tempol (100 mg/kg) plus LPS showed significant decrease of A β content when compared with LPS received Alzheimer group. (Table 1).

Table 1: Effect of Tempol on brain levels of A β in mice with LPS induced amyloidogenesis, oxidative stress

Group Parameter	Normal Control	LPS Control	Tempol (100 mg/kg)
A β (pg/g)	3.2 \pm 0.21	15.7 \pm 0.72 ^a	7.13 \pm 0.31 ^{ab}

Each value represents a mean of 10-15 values \pm SEM.

^asignificantly different from normal control value at $p < 0.05$.

^bsignificantly different from LPS control value at $p < 0.05$.

A β : beta amyloid, LPS: lipopolysaccharide (0.8 mg/kg), SEM: standard error of the mean.

Oxidative stress markers

Lipopolysaccharide (0.8 mg/kg) received group showed significant increase of MDA and significant decrease of GSH and SOD when compared with normal control group, while tempol (100 mg/kg) plus LPS received group showed significant decrease of MDA and significant increase of SOD and GSH when compared with LPS received Alzheimer disease. (table 2).

Table 2: Effect of tempol on oxidative stress markers in mice with LPS induced amyloidogenesis, oxidative stress

Group Parameter	Normal Control	LPS Control	Tempol (100 mg/kg)
SOD (u/g)	8.23 \pm 0.38	2.9 \pm 0.15 ^a	5.4 \pm 0.24 ^{ab}
MDA (nmol/g)	11.2 \pm 0.57	67.5 \pm 2.68 ^a	28.6 \pm 0.95 ^{ab}
GSH (μ mol/g)	5.45 \pm 0.20	0.85 \pm 0.035 ^a	2.33 \pm 0.053 ^{ab}

Each value represents a mean of 10-15 values \pm SEM.

^aSignificantly different from normal control value at $p < 0.05$.

^bSignificantly different from LPS control value at $p < 0.05$.

LPS: lipopolysaccharide (0.8 mg/kg), GSH: glutathione reduced, MDA: Malondialdehyde, lipopolysaccharide, SEM: standard error of the mean, SOD: superoxide dismutase.

DISCUSSION

In the present study, the possible protective effects of tempol, as well as the underlying protective mechanisms, were investigated on LPS-induced amyloidogenesis and neuroinflammation in mice. In this study, memory impairment in mice was associated with brain A β deposition accompanied with massive neurodegenerative changes. In support, LPS administration was reported to enhance brain A β accumulation through pro-



inflammatory potential or through affecting brain permeability.^{25,26}

The deposited A β was reported to be included in activation of free radical generation through activation of NADPH oxidase complex, causing increased rate of ROS formation and lipid peroxidation.⁹⁻¹¹

Additionally, iron and copper ions attached to A β plaques act as catalysts for further ROS formation.^{27,28} Immunologically, microglia cells recognize and attack A β plaques, again increasing cytokine production and causing excessive ROS formation²⁹ as evident in our results, where brain tissue MDA was significantly increased while brain tissue GSH was significantly depleted, which also came in agreement with our results. In support,¹⁰ reported that tempol could decrease cerebral amyloid angiopathy and A β deposition in animal models, Tempol was reported to possess antioxidant effect, being a ROS scavenger^{15,16}, the antioxidant effect of tempol in the present investigation was evidenced by suppressed tissue MDA level, coupled with elevated GSH levels. The recorded ability of tempol in the current study to upgrade SOD activity in brain tissue may explain partly its antioxidant potential. Support of antioxidant status was claimed by many authors to suppress AD progression in different models.³⁰⁻³² Similarly, oxidative stress in brain tissue was considered as a result of A β deposition³³ and also as a pathogenic factor enhancing neuronal damage and AD progression.³⁴ The antioxidant effect of tempol and telmisartan may therefore represent another explanation to the beneficial anti-Alzheimer effect of tempol evident in the current work.

CONCLUSION

Lipopolysaccharide (0.8 mg/kg) induced amyloidogenesis and neuroinflammation and neuronal degeneration including oxidative stress. Tempol at dose of 100 mg/kg could improve neuroinflammation and neuronal degeneration through significant decrease of amyloid β deposition in neurons and significant decrease oxidative stress parameters through significant decrease of MDA and significant increase of GSH and SOD.

REFERENCES

- Lee Y.J, Choi DY, Choi IS, Kim KH, Kim YH, Kim HM, Lee K, Cho WG, Jung JK, Han SB, Inhibitory effect of 4-O-methylhonokiol on lipopolysaccharide-induced neuroinflammation, amyloidogenesis and memory impairment via inhibition of nuclear factor-kappaB *in vitro* and *in vivo* models, *J Neuroinflammation*, 9, 2012, 35.
- Mattson MP, Maudsley S, Martin B, BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders, *Trends in neurosciences*, 27, 2004, 589-594.
- Francis PT, Palmer AM, Snape M, Wilcock GK, The cholinergic hypothesis of Alzheimer's disease: a review of progress, *Journal of Neurology, Neurosurgery & Psychiatry*, 66, 1999, 137-147.
- Buckingham SD, Jones AK, Brown LA, Sattelle DB, Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection, *Pharmacological Reviews*, 61, 2009, 39-61.
- Lombardo S, Maskos U, Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment, *Neuropharmacology* 2014.
- Mudher A, Lovestone S, Alzheimer's disease–do tauists and baptists finally shake hands? *Trends in neurosciences*, 25, 2002, 22-26.
- Boutajangout A, Wisniewski T, Tau-Based Therapeutic Approaches for Alzheimer's Disease-A Mini-Review, *Gerontology*, 60, 2014, 381-385.
- Tran L, Ha-Duong T, Exploring the Alzheimer amyloid- β peptide conformational ensemble: A review of molecular dynamics approaches, *Peptides*, 69, 2015, 86-91.
- Hardy J, Selkoe DJ, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, *Science*, 297, 2002, 353-356.
- Han BH, Zhou M, Johnso AW, Singh I, Liao F, Vellimana AK, Nelson JW, Milner E, Cirrito JR, Basak J, Contribution of reactive oxygen species to cerebral amyloid angiopathy, vasomotor dysfunction, and microhemorrhage in aged Tg2576 mice, *Proceedings of the National Academy of Sciences*, 112, 2015, E881-E890.
- Mota SI, Costa RO, Ferreira IL, Santan I, Caldeira GL, Padovano C, Fonseca AC, Baldeiras I, Cunha C, Letra L, Oxidative stress involving changes in Nrf2 and ER stress in early stages of Alzheimer's disease, *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1852, 2015, 1428-1441.
- Baranello R, Bharani K, Padmaraju V, Chopra N, Lahiri D, Greig N, Pappolla M, Sambamurti K, Amyloid-beta protein clearance and degradation (ABCD) pathways and their role in Alzheimer's disease, *Current Alzheimer Research*, 12, 2015, 32-46.
- Carroll RT, Galatsis P, Borosky S, Kopec KK, Kumar V, Althaus JS, Hall ED, 4-Hydroxy-2, 2, 6, 6-tetramethylpiperidine-1-oxyl (Tempol) inhibits peroxynitrite-mediated phenol nitration, *Chemical research in toxicology*, 13, 2000, 294-300.
- Liang Q, Smith AD, Pan S, Tyurin VA, Kagan VE, Hastings TG, Schor N F, Neuroprotective effects of TEMPOL in central and peripheral nervous system models of Parkinson's disease, *Biochemical pharmacology*, 70, 2005, 1371-1381.
- Wilcox CS, Pearlman A, Chemistry and antihypertensive effects of tempol and other nitroxides, *Pharmacological reviews*, 60, 2008, 418-469.
- Mizera R, Hodyc D, Herget J, ROS scavenger decreases basal perfusion pressure, vasoconstriction and NO synthase activity in pulmonary circulation during pulmonary microembolism, *Physiological research/Academia Scientiarum Bohemoslovaca*, 2015.
- Arai K, Matsuki N, Ikegaya Y, Nishiyama N, Deterioration of spatial learning performances in lipopolysaccharide-treated mice. *The Japanese Journal of Pharmacology*, 87, 2001, 195-201.



18. Nunomura A, Castellani RJ, Zhu X, Moreira P I, Perry G, Smith MA, Involvement of oxidative stress in Alzheimer disease, *Journal of Neuropathology & Experimental Neurology*, 65, 2006, 631-641.
19. E Abdel Moneim A, Oxidant/Antioxidant Imbalance and the Risk of Alzheimer's Disease, *Current Alzheimer Research*, 12, 2015, 335-349.
20. Sheng JG, Bora SH, Xu G, Borchelt DR, Price DL, Koliatsos VE, Lipopolysaccharide-induced-neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid β peptide in APPswe transgenic mice. *Neurobiology of disease*, 14, 2003, 133-145.
21. Verges DK, Restivo JL, Goebel WD, Holtzman DM, Cirrito JR, Opposing synaptic regulation of amyloid- β metabolism by NMDA receptors *in vivo*. *The Journal of Neuroscience*, 31, 2011, 11328-11337.
22. Robak J, Gryglewski RJ, Flavonoids are scavengers of superoxide anions, *Biochemical pharmacology*, 37, 1988, 837-841.
23. Ohkawa H, Ohishi N, Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Analytical biochemistry*, 95, 1979, 351-358.
24. Ellman GL, Tissue sulfhydryl groups, *Archives of biochemistry and biophysics*, 82, 1959, 70-77.
25. Jaeger LB, Dohgu S, Sultana R, Lynch JL, Owen JB, Erickson MA, Shah GN, Price TO, Fleegal-Demotta MA, Butterfield DA, Lipopolysaccharide alters the blood-brain barrier transport of amyloid β protein: A mechanism for inflammation in the progression of Alzheimer's disease, *Brain, behavior, and immunity*, 23, 2009, 507-517.
26. Maher A, El-Sayed N S E, Breitinger HG, Gad MZ, Overexpression of NMDAR2B in an inflammatory model of Alzheimer's disease: Modulation by NOS inhibitors, *Brain research bulletin*, 109, 2014, 109-116.
27. Collingwood JF, Chong RK, Kasama T, Cervera-Gontard L, Dunin-Borkowski RE, Perry G, Posfai M, Siedlak SL, Simpson ET, Smith MA, Three-dimensional tomographic imaging and characterization of iron compounds within Alzheimer's plaque core material, *Journal of Alzheimer's Disease*, 14, 2008, 235-246.
28. Graham SF, Nasaruddin MB, Carey M, Holscher C, McGuinness B, Kehoe PG, Love S, Passmore P, Elliott CT, Meharg AA, Age-associated changes of brain copper, iron and zinc in Alzheimer's disease and dementia with Lewy bodies, *Journal of Alzheimer's disease: JAD*, 42, 2014, 1407-1413.
29. Gallagher JJ, Finnegan ME, Grehan B, Dobson J, Collingwood JF, Lynch MA, Modest amyloid deposition is associated with iron dysregulation, microglial activation, and oxidative stress. *Journal of Alzheimer's disease*, 28, 2012, 147.
30. Braidy N, Zarka M, Welch J, Bridge W, Therapeutic Approaches to Modulating Glutathione Levels as a Pharmacological Strategy in Alzheimer's Disease, *Current Alzheimer Research*, 12, 2015, 298-313.
31. Leirós M, Alonso E, Rateb ME, Houssen WE, Ebel R, Jaspars M, Alfonso A, Botana L M, Gracilins: Spongionella-derived promising compounds for Alzheimer disease, *Neuropharmacology*, 93, 2015, 285-293.
32. Persichilli S, Gervasoni J, Di Napoli A, Fuso A, Nicolia V, Giardina B, Scarp S, Desiderio C, Cavallaro RA, Plasma Thiols Levels in Alzheimer's Disease Mice under Diet-Induced Hyperhomocysteinemia: Effect of S-Adenosylmethionine and Superoxide-Dismutase Supplementation, *Journal of Alzheimer's disease: JAD*, 44, 2015, 1323-1331.
33. Kim EA, Cho C, Kim D, Choi S, Huh JW, Cho SW, Antioxidative effects of ethyl 2-(3-(benzo [d] thiazol-2-yl) ureido) acetate against amyloid β -induced oxidative cell death via NF- κ B, GSK-3 β and β -catenin signaling pathways in cultured cortical neurons, *Free radical research*, 49, 2015, 411-421.
34. Dubinina E, Schedrina L, Neznanov N, Zalutskaya N, Zakharchenko D, [Oxidative stress and its effect on cells functional activity of alzheimer's disease], *Biomeditsinskaja khimiia*, 61, 2015, 57-69.

Source of Support: Nil, Conflict of Interest: None.

