Preetha selva and Seetha Lakshmi S. / Vol 3 / Issue 2/ 2013 / 51-54.

International Journal of
Innovative Drug Discovery
e ISSN 2249 - 7609
Print ISSN 2249 - 7617
www.ijidd.com

ANTI-OXIDANT ACTIVITY OF MINOCYCLINE- AN *IN VITRO* STUDY

Preetha Selva and Seetha Lakshmi S

Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Porur, Chennai-600116, India.

ABSTRACT

Minocycline is a semi synthetic second generation tetracycline. Apart from antimicrobial activity, it also plays a neuroprotective role in many animal models of acute CNS injuries and neurodegenerative diseases. However, the molecular basis for neuroprotective effects of minocycline remains unclear. In this study, we have evaluated the antioxidant properties of minocycline using the *in vitro* DPPH assay. This drug was found to have significant antioxidant capacity which could also be a cause for its neuroprotective role and hence can be used in a wide range of conditions.

KEY WORDS: Minocycline, DPPH, Neuroprotective, Antioxidant activity.

INTRODUCTION

Minocycline is a broad-spectrum tetracycline antibiotic, and has a broader spectrum than the other members of the group. It is a bacteriostatic antibiotic, classified as a long-acting type. Minocycline is the most lipid-soluble of the tetracycline-class antibiotics, giving it the greatest penetration into the prostate and brain. Minocycline is not a naturally-occurring antibiotic, but was synthesized semi-synthetically from natural tetracycline antibiotics by Lederle Laboratories in 1972. Apart from antimicrobial property, minocycline is also said to play a neuroprotectiverole in many animal models. It is neuroprotective in rodent models of cerebral ischemia when dosed up to 4–5 h post-ischemia [1-2]. Minocycline is also effective in other acute CNS injury and neurodegenerative disease models, including the MPTP mouse model of Parkinson's disease [3-4] transgenic mouse models of amyotrophic lateral sclerosis [5-6] and Huntington's disease [7] and a rat model of spinal cord injury [8-9]. Given minocycline's promising efficacy in preclinical models, and given that it is a generally safe and well-tolerated drug, it is important to understand the mechanism for its neuroprotective effects.

Many proposed mechanisms of action are associated with minocycline-mediated neuroprotection. The neuroprotective action of minocycline may include its inhibitory effect on 5-lipoxygenase [10], an inflammatory enzyme associated with brain aging, and the antibiotic is being studied for use in Alzheimer's disease patients [11]. Minocycline also affects expression levels of the anti-apoptotic protein Bcl-2 and the activity of the pro-apoptotic protein Bid, consequently preventing activation of caspases 3, 8 and 9 [12-14]. In cell culture studies, minocycline has been shown to inhibit cell death induced by a variety of insults including NMDA, thapsigargin, hydrogen peroxide, nitric oxide, and 6-hydroxydopamine. In cells treated with toxic levels of nitric oxide, protection by minocycline was associated with inhibition of p38 activation. In cultured rat cerebellar granule neurons (CGNs) treated with 6-hydroxydopamine, minocycline attenuated free-radical production and cell death [15-16].

There is considerable evidence for neuroprotective effects of antioxidants, particularly in animal models of stroke and Parkinson's disease [17]. The fact that minocycline is effective in animal models of stroke and Parkinson's disease, together with evidence that minocycline has free-radical scavenging activity [18], suggested to us that minocycline's direct antioxidant properties may play a role in its mechanism of neuroprotection. In this present study, the free radical scavenging activity of minocycline was evaluated using the *invitro* DPPH assay.

Corresponding Author:- **Preetha Selva** Email:- drpreethaselva@gmail.com

MATERIALS AND METHODS

Chemicals

DPPH (1,1-diphenyl, 2-picrylhydrazyl) and minocycline was obtained from Sigma Chemical Co. CRC, bangalore. Ascorbic acid was obtained from SD Fine Chem. Ltd., Biosar, India.

DPPH free radical scavenging activity

The free radical scavenging capacity of minocycline was determined using DPPH [19]. DPPH solution (0.004% w/v) was prepared in 95% methanol. Sample was mixed with 95 % methanol to prepare the stock solution (100µg/ml). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and minocycline was added in serial dilutions (20 µg to 120 µg) to every test tube so that the final volume was 3 ml and after 15 min of incubation in dark at room temperature, the absorbance was read at 517 nm using aspectrophotometer (HACH 4000 DU UV–visible spectrophotometer). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (100µg/ml). Control sample was prepared containing the same volume. 95 %methanol served as blank.

% scavenging of the DPPH free radical was measured using the formula:

% DPPH radical scavenging activity was calculated using the formula = (Ac-As (or) At)/Ac X100

Where Ac is absorbance of control, As is absorbance of standard or test.

IC50 values were obtained by probit analysis [20].

Figure 2: Test tubes containing varying concentrations of minocycline and ascorbic acid with freshly prepared DPPH solution

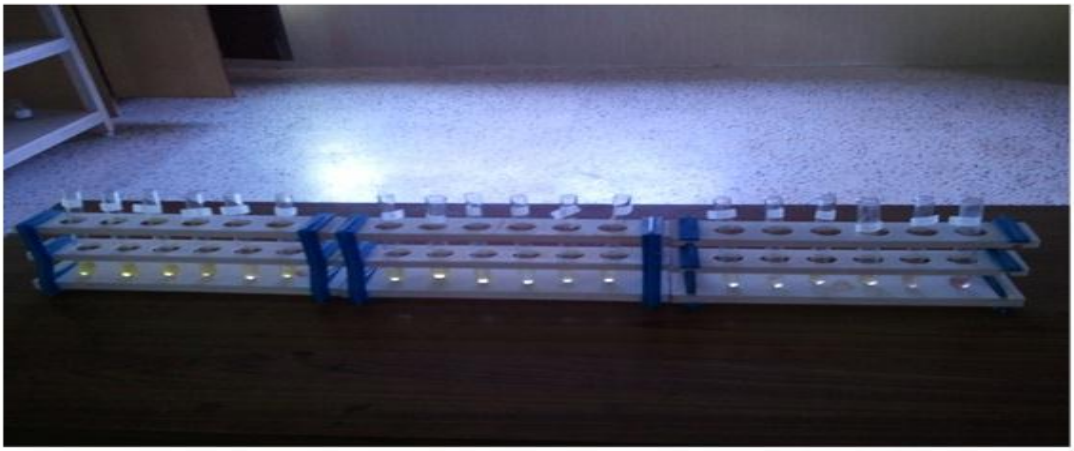


Fig3. Absorbance of minocycline and ascorbic acid at increasing concentrations

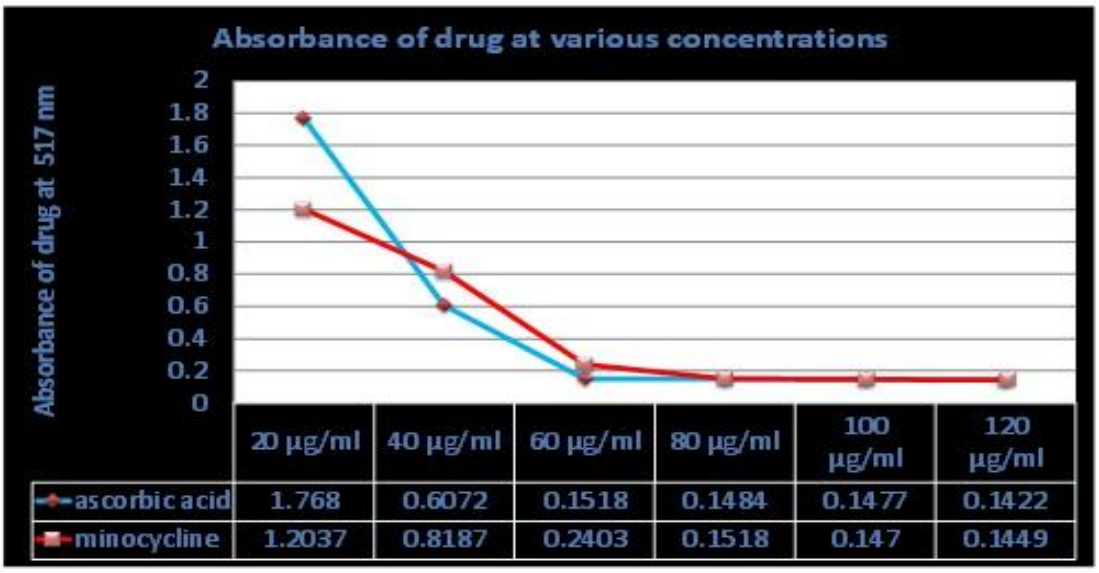
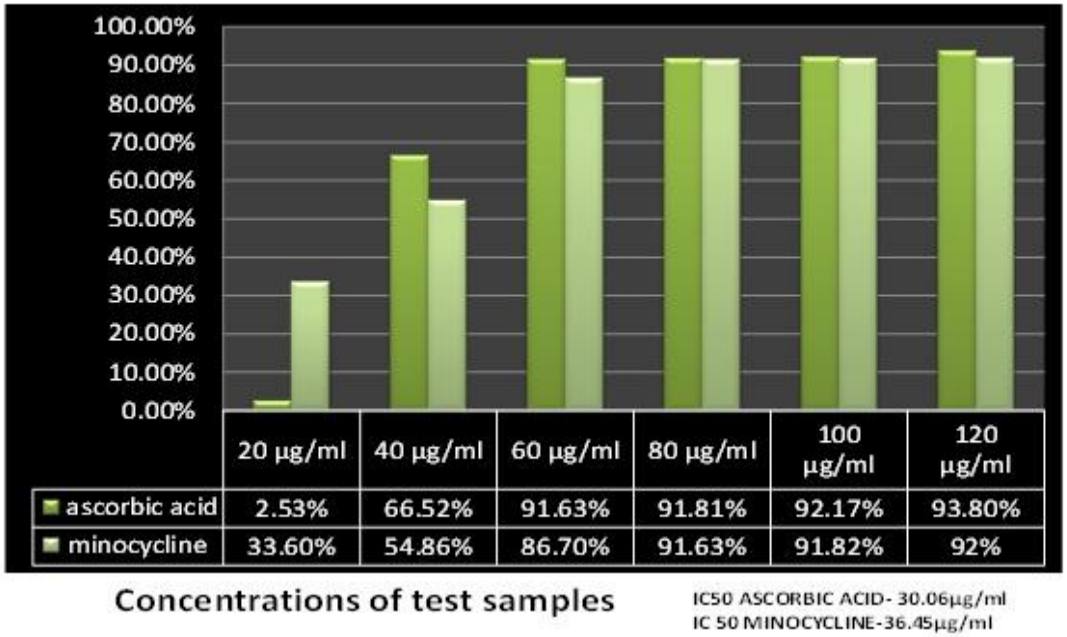


Fig 4. DPPH free radical scavenging assay of minocycline and ascorbic acid



RESULTS AND DISCUSSION

At concentrations of 20, 40, 60, 80, 100 and 120 µg/ml the percentage of inhibition of the drug minocycline was 33.6%, 54.86%, 86.7% 91.63%, 91.82% and 92% respectively. The percentage of inhibition of the standard antioxidant (ascorbic acid) at the same concentrations were 2.53%, 66.52%, 91.63%, 91.81%, 92.17% and 93.8% respectively. The IC50 value for ascorbic acid was 30.06µg/ml and IC 50 value of minocycline was 36.45µg/ml.

CONCLUSION

From our study, the free radical scavenging property as measured by DPPH method showed that percentage of inhibition increases with increasing concentrations of minocycline.It is an effective antioxidant with radical scavenging potency almost similar to vitamin C.Hence minocycline could play a role of a neoadjuvant antioxidant in a wide range of conditions.

ACKNOWLEDGEMENTS

I gratefully acknowledge the Head of the Department of Pharmacology Dr. S.Seethalakshmi who helped me to carry out this research work.

REFERENCES

1. Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH *and* Koistinaho J. *(*1999*)* A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc. Natl Acad. Sci. USA*, (13), 1999, 496–13 500.
2. Xu L, Fagan SC, Waller JL, Edwards DJ, Borlongan CV, Zheng J, Hill WD, Feuerstein G *and* Hess DC. Low dose intravenous minocycline is neuroprotective after middle cerebral artery occlusion-reperfusion in rats. *BMC Neurol*. 2004, 4.
3. Du Y, Ma Z, Lin S *et al.,* Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proc. Natl Acad. Sci. USA*, 98, 2001, 14 669– 674.
4. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi DK, Ischiropoulos H *and* Przedborski S. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J. Neurosci*, 22, 2002, 1763–1771.
5. Van Den BL, Tilkin P, Lemmens G *and* Robberecht W. Minocycline delays disease onset and mortality in a transgenic model of ALS. *Neuroreport*, 13, 2002, 1067–1070.
6. Zhu S, Stavrovskaya IG, Drozda M *et al.,* Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature*, 417, 2002, 74–78.
7. Chen M, Ona VO, Li M *et al.,* Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat. Med*, 6, 2000, 797–801.

8. Stirling DP, Khodarahmi K, Liu J, McPhail LT, McBride CB, Steeves JD, Ramer MS *and* Tetzlaff W. Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. *J. Neurosci*, 24, 2004, 2182–2190.
9. Teng YD, Choi H, Onario RC, Zhu S, Desilets FC, Lan S, Woodard EJ, Snyder EY, Eichler ME *and* Friedlander RM. Minocycline inhibit contusion-triggered mitochondrial cytochrome c release and mitigates functional deficits after spinal cord injury. *Proc. Natl Acad. Sci. USA*, 101, 2004, 3071–3076.
10. Song Y, Wei EQ, Zhang WP, Zhang L, Liu JR, Chen Z. Minocycline protects PC12 cells from ischemic-like injury and inhibits 5-lipoxygenase activation. *NeuroReport*, 15(14), 2004, 2181–4.
11. Uz T, Pesold C, Longone P, Manev H. Aging-associated up-regulation of neuronal 5-lipoxygenase expression: putative role in neuronal vulnerability. *FASEB J*, 12(6), 1998, 439–49.
12. Friedlander RM. Apoptosis and caspases in neurodegenerative diseases. *N. Engl. J. Med.* 2003; 348: 1365–1375.
13. Blum D, Chtarto A, Tenenbaum L, Brotchi J *and* Levivier M. Clinical potential of minocycline for neurodegenerative disorders. *Neurobiol. Dis*, 17, 2004, 359–366
14. Domercq M. *and* Matute C. Neuroprotection by tetracyclines. *Trends Pharm. Sci*, 25, 2004, 609–612
15. Lin S, Wei X, Xu Y, Yan C, Dodel R, Zhang Y, Liu J, Klaunig JE, Farlow M *and* Du Y. Minocycline blocks 6-hydroxydopamine-induced neurotoxicity and free radical production in rat cerebellar granule neurons. *Life Sci.*, 72, 2003, 1635–1641.
16. Lin S, Zhang Y, Dodel R, Farlow MR, Paul SM *and* Du Y. Minocycline blocks nitric oxide-induced neurotoxicity by inhibition p38 MAP kinase in rat cerebellar granule neurons. *Neurosci.Lett.*, 315, 2001, 61–64.
17. Gilgun-Sherki Y, Rosenbaum Z, Melamed E *and* Offen D. Antioxidant therapy in acute central nervous system injury: current state. *Pharmacol. Rev*, 54, 2002, 271–284.
18. Miyachi Y, Yoshioka A, Imamura S *and* Niwa Y. Effect of antibiotics on the generation of reactive oxygen species. *J. Invest. Dermatol*, 86, 1986, 449–453.
19. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int. J. Biomed.Sci*, 4, 2008, 89-96.
20. Viturro C, Molina A and Schmeda-Hirschmann G. Free radical scavengers from *Mutisia friesiana* (Asteraceae) and *Sanicula graveolens* (Apiaceae). *Phytother. Res*, 13, 1999, 422-424.