CHAPTER 6

CONCLUSION AND SCOPE FOR FUTURE WORK

6.1. Summary and Conclusion

Evidences have shown that RAS plays a major role in both glial and neuronal functions. Existence of intense hyperactivated glial cells such as astrocytes and microglia are major hallmarks in the pathophysiology of PD. This shows that restoration of dopaminergic functions alone may not be sufficient in the management of PD. The present study demonstrates the neuroprotective effects of Telmisartan (TEL) in MPTP intoxicated acute and chronic mice models of Parkinson's disease, with respect to the glial-neuronal functions.

In the acute model, C57BL/6J mice were intoxicated with MPTP at two dose regimens (2 X 40 mg/kg i.p., 16 h apart and sacrificed 48 h after first injection; 4 X 20 mg/kg i.p., 4 h apart and sacrificed after one month) to induce neurodegeneration. Mice administered with MPTP at 2 X 40 mg/kg i.p., 16 h apart and sacrificed 48 h after first injection showed intense and uniform pattern of neurodegeneration when compared to the other dose regimen. Hence, this protocol was adopted to study the effect of TEL on acute MPTP intoxication.

In the acute MPTP model, TEL improved motor functions, which corroborates to the increased dopamine, DOPAC and HVA levels. On the other hand, TEL decreased α-syn aggregation and increased neurotrophic factors such as BDNF and GDNF. In addition, TEL also improved neuronal markers expressions such as DAT, TH and VMAT2 and decreased astroglial marker GFAP in MPTP intoxicated mice brain.
In chronic MPTP model, TEL down-regulated \(iNOS\) expression in turn NO level in MPTP intoxicated mice brain. It up-regulated GDNF and down-regulated GFAP and \(\alpha\)-syn expressions. TEL decreased IL-1\(\beta\) and TNF\(\alpha\), which correlates with decreased GFAP expression. TEL balances NO and GSH levels, which could be the possible reason for increased DAT, VMAT2, TH and NeuN count. The increased dopamine and its metabolites turnover with TEL administration reflect the increased TH expression. Increased NeuN count with TEL treatment reveals its neuroproective effects which was further evidenced with presence of Nissl bodies in histopathological examination. TEL improved motor function which corroborates to increased dopamine content.

Correlation analysis also revealed that glial markers such as GDNF and GFAP possess strong positive and negative correlation with neuronal markers – DAT, TH, VMAT2, NeuN, Nissl bodies, dopamine, DOPAC and HVA respectively in MPTP intoxicated mice brain. Dopamine exhibited strong positive correlation with motor functions which was evidenced from beam walk, gait, vertical and horizontal grid test. This shows that TEL has the ability to recover motor functions in PD which could be due to improvement in dopamine levels.

In conclusion,

- Acute MPTP study highlights the neuroprotective effect of TEL against MPTP induced neurodegeneration in C57BL/6J mice. TEL exerts its protective effect through regulation of \(\alpha\)-syn and neurotrophic factors such as BDNF and GDNF in MPTP intoxicated mice.
- Chronic MPTP study highlights that TEL exerts its neuroprotection through the regulation of astrocytic components such as \(iNOS\), NO, GDNF, GFAP and GSH and dopaminergic components such as DAT, TH and dopamine levels as well in MPTP intoxicated mice.
- The present study establishes the neuroprotective effects of TEL in an acute and chronic model of Parkinsonism with respect to the astrocytic - dopaminergic functions and adds evidence to the disease - modifying therapeutic potential of central ATIR antagonism.
6.2. Scope for future work

- Therapeutic intervention with AT1R antagonists in a fully blown Parkinsonism state in genetic mouse and evaluation of clear mechanism of action
**Fig. 6.1. Molecular mechanism of neurodegeneration on MPTP intoxication.**

MPTP, a highly lipophilic pro-toxin, crosses the blood-brain barrier and reaches astrocytes. In astrocytes, it gets converted into MPP⁺ by MAO-B enzyme. MPP⁺ activates iNOS synthesis which results in the overproduction of NO and finally depletion of GSH. MPP⁺ enters dopaminergic neurons through DAT and reaches cytosol. MPP⁺ oxidizes cytosolic proteins and forms protein aggregates, which in turn activates GFAP and astrocytosis as a feedback mechanism. It enters synaptic vesicles through VMA22 and oxidizes dopamine resulting in dopamine depletion. MPP⁺ blocks mitochondrial complex I which results in depletion of ATP and overproduction of ROS. In addition, MPP⁺ activates Aβ1 receptors in both astrocytes and neurons thus stimulate cytokines and ROS, leading to apoptosis and neurodegeneration.

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP⁺, 1-methyl-4-phenylpyridinium; MAO-B, Monoamine oxidase B; iNOS, inducible nitric oxide synthase; NO, Nitric oxide; GSH, Reduced glutathione; γ GT, γ-glutamyltranspeptidase; Glu, Glutamate; GABA, Glutamine. Cys, Cysteine; AT1R, Angiotensin II type 1 receptor; ROS, Reactive oxygen species; α-syn, α-synuclein; DAT, Dopamine transporter; VMA22, Vesicular monoamine transporter 2; ETC, Electron transport chain.
Fig. 6.2. Proposed mechanism of action of TEL against MPTP induced neurodegeneration.

TEL, an AT1R blocker, ameliorates MPTP induced alterations in both astrocytes and dopaminergic neurons. TEL binds with AT1R and reduces cytokines production. Decreased cytokines results in the suppression of NOS and NO production. This results in the restoration of GSH content. TEL also reduces MPP+ induced dopamine depletion and complex I blockage which results in decreased ROS production and restores dopaminergic neurons.

TEL: Telmisartan; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP+: 1-methyl-4-phenylpyridinium; MAO-B: Monoamine oxidase B; NOS: inducible nitric oxide synthase; NO: Nitric oxide; GSH: Reduced glutathione; GGT: γ-glutamyltransferase; Glu: Glutamate; Gly: Glycine; Cys: Cysteine; AT1R: Angiotension II type 1 receptor; ROS: Reactive oxygen species; α-synt: α-synuclein; DAT: Dopamine transporter; VMAT2: Vesicular monoamine transporter 2; ETC: Electron transport chain.