

EVALUATION OF SOME MITOCHONDRIAL MODULATORS IN TREATMENT OF NEONATAL ANOXIA



Thesis submitted in partial fulfillment for the
Award of Degree of
Doctor of Philosophy

By

Mr. Puneet K Samaiya

M.Pharm

DEPARTMENT OF PHARMACEUTICS
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI- 221005
INDIA

Roll No. 13161010

Year 2017

8.1 Summary and Conclusion

The overall study was based on targeting mitochondrial bioenergetics which is progressively lost post-anoxia injury in developing brain of anoxic newborns. We have proposed that mitochondria may be a novel target and has sufficient window of opportunity in treating anoxia by preventing further apoptotic cell death in cortical neurons using a standardized two episodic model of anoxia. Neural development of cortical region is important for sensorimotor development in early days life and neurobehavioral development like memory and cognition throughout life. We also report that there is an existence of primary insult soon after anoxia (i.e. 30 min) along with mitochondrial dysfunction and apoptosis which progresses to secondary insult (day-7) if left untreated, this can cause long sensorimotor and other neurobehavioral impairments. Further, we report the expression of mitochondrial apoptotic markers like cytochrome-C, caspase-9/3 in the CSF of neonates in a clinical study. This indicates the increased levels of mitochondrial apoptotic markers (cytochrome-C, caspase-9/3) in asphyxic newborns. Further, the levels of these biomarkers increased with the severity of HIE insult. In this context, we hypothesized that mitochondrial modulators like 2,4 DNP and tempol can be a potential pharmacological entities which can reverse mitochondrial dysfunction and rescue from further insult progression. Moreover we have proven our hypothesis which states that (1) mild uncoupling through 2,4 DNP can inhibit Ca^{2+} cycling/overloading and can preserve mitochondrial function post anoxia. Moreover, the pharmacological effects of 2,4 DNP on mitochondrial bioenergetics was accompanied by a decrease in MMP and inhibition of ROS which resulted in the restoration of mitochondrial function, complex enzyme activity, inhibition of expression of pro-apoptotic mitochondrial Bax. This led to decrease in mitochondrial MPT pore opening and resultant release of

SUMMARY AND CONCLUSION

cytochrome-C. These events ultimately caused inhibition of mitochondrial linked apoptosis by reduced activation of caspase-9 and caspase-3. Overall 2,4 DNP improved the sensorimotor performance indicating improved neurobehavioral outcome and imparting its role to be a potent pharmacological agent to treat anoxia (Figure 1).

Further we hypothesized that (2) use of NO scavenger would be another target to improve mitochondrial function after anoxia. The effects of tempol (75 mg/kg, 150 mg/kg and 300 mg/kg) on mitochondrial bioenergetics were accompanied by scavenging NO and further restoring mitochondrial function, complex enzyme activity, MMP and MPT. Tempol probably acts by mimicking antioxidant activity and inhibiting nitric oxide formation which is a major component in peroxynitrite synthesis. Further, tempol significantly downregulated the expression of pro-apoptotic mitochondrial, Bax. This could lead to prevention of mitochondrial outer membrane permeabilization (MOMP). These events attenuated the release of cytochrome-C and inhibited the expression of caspase-9 and caspase-3 and thereby the progression of apoptosis. Overall tempol improved the neurobehavioral performance indicating improved neurobehavioral outcome after anoxia (Figure 1). Therefore, the study indicates the preclinical potential of tempol for the treatment of anoxia. Furthermore to observe any synergism or additive effect of mitochondrial targets we combined 2,4 DNP and tempol to treat synaptic and non-synaptic mitochondria after anoxia. It was observed that combination of 2,4 DNP and tempol (D+T) did not show any synergetic or additive activity. Further to observe the effect of above combination of the drugs in long-term neurobehavioral performances we evaluated OFT, EPM, Y-maze, FST on d-21,d-60,d-90,d-120 and d-150. Further, the plasma corticosterone levels were measured on day-150 to observe HPA axis activity after anoxia.

SUMMARY AND CONCLUSION

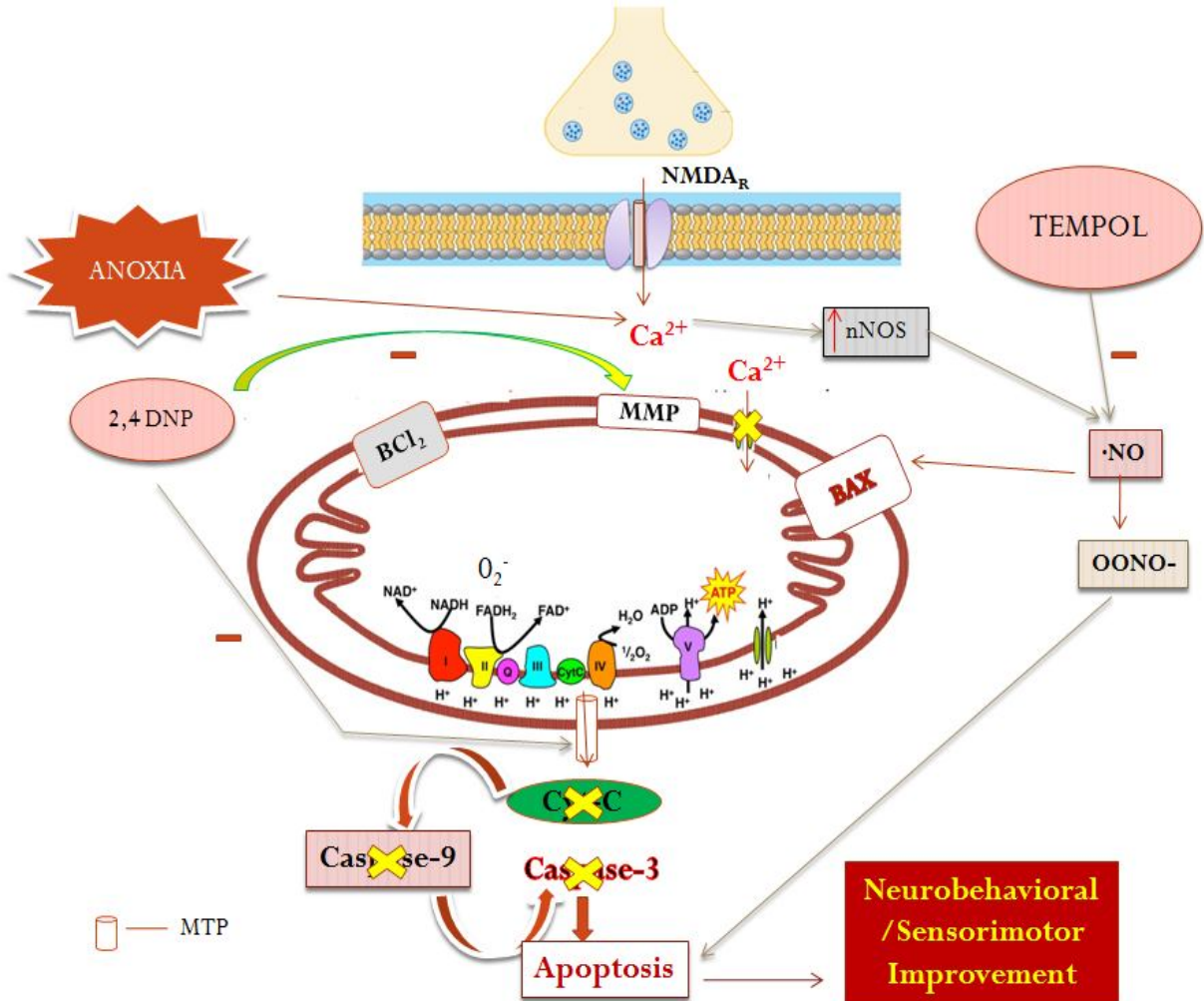


Figure 8.1 Proven hypothesis. “—” denotes inhibition

The individual drugs and the combination of D+T were significantly effective in correcting long term hyperactivity such as spontaneous locomotor activity in OFT, spatial recognition memory in Y-maze and immobility period in FST. However there was no synergistic long term action with combination of D+T. Similarly, there was decrease in anxiety index in EPM paradigm. Overall these results indicated a marked long-term improvement in neurobehavioral performances after anoxia. Furthermore on d-150 after the behavioral parameters assessment, the animals were killed and plasma corticosterone was measured to

SUMMARY AND CONCLUSION

elucidate HPA axis activity. It was observed that the corticosterone levels were significantly decreased after chronic stress due to HPA axis hyperactivity. Treatment with 2,4 DNP, tempol and the combination of D+T was equally effective in improving the corticosterone levels in rats. However there was no synergistic long term action on levels of corticosterone with combination of D+T. Therefore the study indicates that 2,4 DNP and tempol improved long term behavior, mitochondrial function, and stress response. However, the combination of 2,4 DNP and tempol did not show any synergistic effect on individual drugs in improving mitochondrial function as well as neurobehavioral outcomes post-anoxia injury.

In conclusion, anoxia-induced mitochondrial dysfunction can be improved by the use of mitochondrial modulators like 2,4 DNP or tempol which imparts their role in decreasing insult progression and further improvement in sensorimotor and neurobehavioral performance after neonatal anoxia. Therefore, the drugs targeting mitochondrial dysfunction after anoxia can be a potential pharmacological intervention to treat anoxia and improve the neurobehavioral measures later in life.