CHAPTER- 6

Okadaic acid induced neurotoxicity leads to central cholinergic dysfunction in rats

1. Introduction

Neurodegenerative disorders, such as AD are often characterized by the degeneration of the cholinergic system. Loss of cholinergic neurons in the brain and reduced cholinergic activity in the hippocampus and a cortical loss of choline acetyltransferase were observed in AD. Loss of the central cholinergic neurons and down-regulation of the neuronal nicotinic acetylcholine receptors are one of the hallmarks of the pathogenesis of AD (Whitehouse et al., 1982). The blockade of the cholinergic system resulted in transient cognitive impairment, while acetylcholinesterase inhibitors enhanced memory functions (Honer et al., 1987). The neurodegenerative process in AD is initially characterized by synaptic damage accompanied by neuronal loss (Perry et al., 1978). Brain tissue from patients with dementia was examined for activities of choline acetyltransferase and acetylcholinesterase activities, which were decreased significantly in demented subjects (Crews and Masliah, 2010). One of the characteristic changes that occur in AD is the loss of memory and the loss of AChE from both cholinergic and noncholinergic neurons in the brain (Small, 1997). Normal brain function depends heavily on acetylcholine an important neurotransmitter in brain and reduced amount of acetylcholine is observed in AD (Wolf and Butcher, 1986).

2. Methods

Acetylcholinesterase (AChE) and α7-nAChR mRNA expression were estimated RT PCR method in (OKA) ICV treated rat brain areas. AChE kinetic study was done by Ellman’s method (1961) at 412 nm wavelength. Acetylcholine level was estimated by colorimetric assay kit according to manufacturer instruction.
3. Results:

3.1. Acetylcholinesterase activity

AChE kinetic study was done by Ellman’s method (1961) at the end of the experiment. There was no significant difference observed in aCSF and control in all the four (cerebellum, striatum, cerebral cortex and hippocampus) brain region, but significant decreased observed (P<0.01) in AChE activity in cerebral cortex and hippocampus of OKA (200ng) treated brain except cerebellum and striatum. Memantine and donepezil treatment significantly restore AChE activity in cortex and hippocampus (P<0.01) in OKA treated rat (Fig.18).

Fig.18. Figure represents both donepezil and memantine was able to restore reduced AChE activity in OKA treated rat. **P< 0.01 vs. control group and *P< 0.01 vs. OKA group.

3.2. AChE mRNA expression

There was no significant difference (P>0.05) observed in the AChE mRNA expression in control and aCSF group. Okadaic acid significantly (P<0.01) decreases AChE mRNA level in brain areas except striatum as compare to control and aCSF group. Whereas, pretreatment with donepezil and memantine significantly (P<0.01) restore AChE mRNA level in OKA treated rat brain (Fig.19).
Fig. 19. A significant decrease in AChE mRNA was observed in striatum, cortex and hippocampus of OKA treated group which was prevented by donepezil and memantine. *P< 0.01 and **P< 0.001 vs. Control group and *P< 0.05 vs. OKA group.

M- Molecular weight marker, 1- control, 2- aCSF, 3- OKA, 4- Memantine and 5- Donepezil
3.3. α7-nAChR mRNA expression

There was no significant difference observed between control and aCSF group. Whereas OKA (ICV) administration caused significantly (P<0.05) decrease in the α7-nAChR mRNA expression in the cortex and hippocampus. OKA induced decreased α7-nAChR mRNA was restored significantly (P<0.05) by treatment with donepezil and memantine in cortex and hippocampus (Fig.20).
Fig. 20. A significant decrease in α7-nAChR mRNA was observed in striatum, cortex and hippocampus of OKA treated group which was prevented by donepezil and memantine. *P< 0.01 and **P< 0.001 vs. Control group and *P< 0.05 and **P< 0.005 vs. OKA group.

3.4. Acetylcholine (ACh) level

OKA (ICV) significantly (P<0.01) decreased acetylcholine level in cortex and hippocampus as compare to control and aCSF group. Whereas, pretreatment with donepezil and memantine significantly (P<0.01) restored acetylcholine level in OKA treated (Fig. 21). There was no significant difference (P>0.05) observed in the acetylcholine in control and aCSF group.

![Fig. 21. OKA significantly decreased choline level as compare to control. The OKA induced decrease was prevented by memantine and donepezil. #P<0.005 vs respective brain region of control and aCSF group and *P<0.05, *P<0.001 vs respective region of OKA group.](image)
4. Discussion

Cholinergic neuronal system has been implicated in the cognitive deficit associated with aging and neurodegenerative disease (Quirion et al., 1986). Therefore, the studies were carried out to check alteration in cholinergic markers in brain areas (cerebellum, striatum, cortex, hippocampus) was corroborated with OKA (ICV) induced memory impairment in rats. We have included ACh level, AChE and α7-nicotinic receptor are an indicators of cholinergic functions. Acetylcholine (ACh) is a neurotransmitter, which involved with synaptic plasticity in learning and short term memory. α7-nicotinic acetylcholine receptors are present in many tissues in the body and are ionotropic receptor which is triggered by the binding of the neurotransmitter ACh. Acetylcholinesterase, also known as AChE or acetylcholine acetylhydrolase, is an enzyme that degrades the neurotransmitter acetylcholine, producing choline and an acetate group. Loss of α7-nAChRs is found in patients with diverse forms of dementia (Court et al., 2000). AChE activity has been reported to be altered in AD patients (Rinne et al., 2003). Normal brain function depends on acetylcholine, an important neurotransmitter in brain, and reduced amount of acetylcholine is observed in AD (Woolf and Butcher, 1986). These cholinergic indicators are important in learning and memory. At present, increase in ACh level by inhibiting AChE activity is the main therapeutic approach for AD. Further, cholinergic nicotinic receptors α7-nAChRs are neuroprotective (Newhouse et al., 2001). It was found that OKA decreased ACh level, AChE activity and down regulates mRNA expression of AChE and α7-nAChR in hippocampus and cortex. It is pertinent to mention that directions of changes in cholinergic indicators are not correlated with each other. We found decreased in ACh level as well as in activity and expression of its metabolizing enzyme AChE, which are contrary to each other. Moreover, upregulation of receptor is expected when the concentration of neurotransmitter is low. However, in this study α7-nAChR was down regulated with decreased ACh level. These observations suggest that the alterations in cholinergic system may not be considered as functional one.

Furthermore, we examined the effect of antidementic drugs donepezil and memantine on OKA induced cholinergic dysfunction in rat brain. Donepezil alleviated the decline in memory impairment. AChE inhibitors such as donepezil, used to increase the levels of
acetylcholine in the synapse, thereby enhancing cholinergic activity in the affected regions of the brain (Birks and Harvey, 2006). We observed that pretreatment with donepezil restored ACh level, AChE and α7-nAChRs mRNA expression and in cortex and hippocampus of OKA treated rat brain. This study indicated that increased mRNA expression might be due to up titration of AChE gene in respect to decreased AChE activity due to inhibitory action of donepezil. Memantine is a moderate-affinity, uncompetitive, voltage-dependent, NMDA-receptor antagonist (Robinson and Keating, 2006) was also used to see the effect on cholinergic function. We observed that pretreatment with memantine restored the acetylcholine level, AChE and α7-nAChRs mRNA expression in cortex and hippocampus of OKA treated rat brain. This preventive effect of memantine on cholinergic function may be due to neuroprotective effect of memantine in OKA induced neurotoxicity. Pretreatment with donepezil upregulated the expression of α7-nAChRs and AChE mRNA in OKA treated rat, which may potentiate AChE, and α7-nAChRs mediated neuroprotective effects.

In conclusion, present study indicates that cholinergic dysfunction is associated with OKA (ICV) induced neurotoxicity in rats, which was prevented by clinically used antidementic drugs.