A study on neuroinflammatory marker in brain areas of Okadaic acid (ICV) induced memory impaired rats

1. Introduction

The evidences emerged in the recent years inferred a close association of neuroinflammation with the pathogenesis of several degenerative neurologic disorders, including Parkinson’s disease and Alzheimer’s diseases (AD) (Mrak et al., 2005, Tarkowski et al., 1999). The generation of free radical reactive nitrogen species (RNS) and Reactive oxygen species (ROS) triggers neuronal damage via activation of microglia (Liu et al., 2003). The activation of microglia releases of proinflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-beta (IL1-β) (Merrill and Benveniste, 1996). Tanaka et al (2006) suggested that pro inflammatory cytokines, oxygen and nitrogen centered free radicals contribute to the neurodegenerative processes. TNF-α and IL1-β are suggested as important mediator in brain pathology of AD (Tan et al., 2007). Murthy, (1999) also suggested a relationship between neuronal injury and NO originated from glial cells. Inducible NOS (iNOS) is not normally expressed in the brain, but inflammatory mediators such as cytokines cause its expression in microglia and astrocytes (Murphy, 2000), and possibly in neurons (Heneka and Feinstein, 2001). Once expressed iNOS produces high levels of NO continuously and iNOS expressing microglia are consistently found in case of neurodegenerative diseases. NO produced by inducible nitric oxide synthase (iNOS) has been reported as a key mediator of glial induced neuronal death (Sarika et al., 2011).

2. Methods

Proinflammatory cytokine like TNFα and ILβ was estimated by using ELISA kits (R&D Systems) according to manufacturer instruction in OKA treated rat brain areas. Cytokines level is expressed as pg of cytokines/mg total protein (Deak et al., 2003). Expression of iNOS and nNOS were estimated by western blotting and RT PCR method.
3. Results

3.1. Estimation of neuroinflammatory markers

3.1.1. Effect of OKA on TNF-α level

There was no significant difference (P>0.05) observed in the TNF-α level in control and aCSF group. OKA significantly (P<0.01) increased TNF-α level in cortex and hippocampus as compared to control and aCSF group. Treatment with donepezil and memantine significantly (P<0.01) prevented the increase in TNF-α level in OKA treated rat (Fig.22).

![Graph showing TNF-α levels in different brain regions](image)

**Fig.22.** Figure represents both donepezil (5mg/kg) and memantine (10mg/kg) was able to reduce elevated TNF-α level in OKA (200ng) treated rat. #P<0.01 vs. control group and *P< 0.01, **P< 0.001 vs. OKA group.
3.1.2. Effect of OKA on IL-1β level

Control and aCSF groups showed no significant difference (P>0.05) in the IL-1β level while OKA significantly (P<0.01) increased IL-1β level in cortex and hippocampus as compared to control and aCSF groups. Donepezil (5mg/kg) and memantine (10mg/kg) significantly reduce (P<0.01) level of IL-1β as compared to OKA treated rat (Fig.23).

![Graph showing IL-1β levels in different brain regions with OKA treatment and interventions](image)

**Fig.23.** OKA significantly increases IL-1β level as compared to control group. Treatment with donepezil and memantine significantly reduced elevated IL-1β level in OKA injected rats. *P< 0.01 vs. aCSF group and *P< 0.01 vs. OKA group.
3.1.3. Total Nitrite level

Nitrite level was significantly (P<0.001) elevated in cortex and hippocampus of OKA treated rat brain in comparison to control and aCSF groups. Pretreatment with donepezil and memantine significantly prevented (P<0.001) this increase in nitrite levels in the brain areas of OKA 200 ng treated rat (Fig.24).

Fig.24. A significant increased in nitrite level was observed in cortex and hippocampus of OKA (200ng) treated group which was reversed by donepezil and memantine. #P< 0.01 and vs. Control group and *P< 0.05, **P< 0.001 vs. OKA group.
3.1.4. iNOS mRNA expression

OKA significantly (P<0.01) increased iNOS mRNA level in cortex and hippocampus as compared to control and aCSF group. No significant difference (P>0.05) was observed in the iNOS mRNA level of aCSF group as compared to control and aCSF treated groups. Pretreatment with donepezil (5mg/kg) and memantine (10mg/kg) significantly (P<0.01) normalize the iNOS level in OKA treated rat (Fig.25).

![Graph showing iNOS mRNA expression](image)

**Fig.25.** A significant increased in iNOS mRNA expression was observed in striatum, cortex and hippocampus of OKA treated group which was prevented by donepezil and memantine. ###P< 0.001 vs. Control group and *P< 0.05 vs. OKA group.
3.1.5. iNOS protein expression

There was no significant difference (P>0.05) observed in the iNOS protein in control and aCSF groups. Whereas OKA significantly (P<0.01) increased iNOS level in cortex and hippocampus as compared to control and aCSF groups. Pretreatment with donepezil (5mg/kg) and memantine (10mg/kg) significantly (P<0.01) restored the iNOS level in OKA treated rat brain regions (Fig.26).

Fig.26. A significant increased in iNOS protein was observed in striatum, cortex and hippocampus of OKA treated group which was normalized by donepezil and memantine. 

#P< 0.01 and ##P< 0.01 vs. Control group *P< 0.05, **P< 0.001 vs. OKA group.
3.1.6. nNOS mRNA expression

OKA significantly decreased (P<0.05) nNOS mRNA expression in the cortex and hippocampus regions while there was no significant difference observed in nNOS mRNA expression of control and aCSF group. Further, there was a significant (P<0.05) increase in nNOS mRNA expression following treatment with donepezil and memantine in OKA injected rat (Fig.27).
**Fig.27.** A significant increased in nNOS mRNA was observed in cortex and hippocampus of OKA treated group which was reversed by donepezil and memantine. *P< 0.01 vs. Control group and *P< 0.05 vs. OKA group.

### 3.1.7. Effect of OKA on nNOS protein expression

ICV administration of aCSF had no significant effect (P>0.05) on nNOS protein expression in comparison to control. However, OKA significantly (P<0.05) decrease the nNOS protein expression in the cortex and hippocampus regions of rat brain as compared to control and aCSF groups. Treatment with donepezil and memantine significantly increased (P<0.05) nNOS protein expression in cortex and hippocampus regions of OKA treated rat (Fig.28).
Fig. 28. A significant increased in nNOS protein was observed in cortex and hippocampus of OKA treated group which was restored by donepezil and memantine. ##P < 0.001  vs. Control group and *P < 0.05 vs. OKA group.

4. Discussion

Neuroinflammation is now being as one of the crucial step in neurodegeneration. Therefore, the studies were performed to find out the involvement of neuroinflammatory changes accompanied with memory impairment following ICV administration of OKA in rats. Release of cytokines TNF-α and IL-1β initiates inflammatory cascades (Frankola et
al. 2011). Therefore, TNF-α and IL-1β are considered as markers of inflammation in peripheral tissue as well as in the brain (Qin et. al., 2008). OKA treatment showed elevated level of cytokine tumor necrosis factor alpha (TNF-α) and interleukin-1 β (IL-1β). The expression of cytokines, free radical generation and neurodegenerative changes are correlated with each other and may contribute to the pathologic process (Fernandez-Botran et al. 2011). The association of free radicals in the neuroinflammation was further investigated in this study.

The oxidative stress has been implicated as a major factor in neurodegeneration and neuroinflammation. The reactive nitrogen and oxygen species are important contributors in activation of microglia, which initiates neuroinflammatory processes by the release of proinflammatory cytokines TNF-α and IL1-β (Liu et al. 2003). In pathological conditions proinflammatory cytokines along with oxygen and nitrogen centered free radicals act as mediator in neuroinflammatory processes (Tanaka et al. 2006; Tan et al. 2007). Pathological conditions in the brain often lead to over-stimulation of the NMDA receptors by glutamate leading to elevation of NO level that may induce excitotoxic neurotoxicity (Seyidova et al. 2004). Rise in nitrite level in rat brain was also observed following ICV OKA administration (Kamat et al. 2010). In the present study level of total nitrite found rose indicating nitrosative stress, i.e. an increase in NO. Nitric oxide (NO) is derived from neuronal nitric oxide synthase (nNOS) and has an important role in neuronal degeneration (Calabrese et. al., 2000). Besides nNOS, an inducible form of NOS (iNOS) also exists but not significantly expressed by resident cells unless cellular activation occurs following that iNOS is produced by several types of cells, such as macrophages, microglia, astrocytes (Simmons and Murphy, 1992). Therefore, inducible NOS (iNOS) is frequently associated with inflammatory conditions. The similar picture appeared in this study. Following OKA administration, expression of iNOS was upregulated while nNOS down regulated in the cortex and hippocampus. The changes in level of nitrite, TNF-α and IL-1β and expression of NOS occurred only in hippocampus and cortex. This observation clearly points out an association between neuroinflammation and nitrosative stress. Present study indicates that iNOS as a source of nitrosative stress may be an important factor in neuroinflammation.
Neuroinflammation is a characteristic feature of both acute and chronic CNS disorders and has been recently implicated in pathogenesis of neurodegenerative dementia (Akiyama et al. 2000). Proinflammatory cytokines-TNF-α and IL-1β released by activated glial cells during brain inflammation have been proposed to contribute to neuropathology underlying cognitive deficits in AD (Reale et al. 2004)). Keeping these facts in view the present study explored the possibility of correlation between neuroinflammatory changes and memory impairment. The neuroinflammation indicated by altered level of RNS and proinflammatory cytokines was evident in cortex and hippocampus but not in cerebellum. As the both affected brain regions are involved in memory function, it could be suggestive of correlation between memory impairment with neuroinflammation. To further correlate neuroinflammation with memory impairment, we investigated the effect of anti-dementic drugs donepezil and memantine on OKA induced neuroinflammatory changes. These drugs prevented alteration in nitrite, nNOS, iNOS, TNF-α and IL-1β expression in cortex and hippocampus of OKA treated rat brain and further substantiated protection against memory impairment. Donepezil is reported to exert protective effect against oxidative stress in streptozotocin induced memory impaired mice (Saxena et al. 2008). Memantine (NMDA receptor antagonist) protects against free radical generation and glutamate excitotoxicity (Seki et al. 2008). In our earlier study both the anti-dementic drugs donepezil and memantine showed significant reduction in free radical generation in different brain areas of OKA treated rats along with memory impairment (Kamat et al. 2010). Therefore, it seems that protective effect of these drugs against OKA induced neuroinflammation may be mediated through suppression of oxidative stress.

Thus, in conclusion it may be suggested that OKA (ICV) induced memory impairment is associated with neuroinflammation in the brain that can be prevented by clinically used antidementic drugs.