



Time Dependent Inflammatory Changes Ensued in Traumatic Brain Injury in Mice

Nidhi Khatri¹, Sunil Sharma^{2*}

^{1,2*}Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science & Technology, Hisar, Haryana

¹Email: nidhikhatri9@gmail.com;

^{2*}Email: sharmask71@rediffmail.com

***Corresponding Author:** - Prof. Sunil Sharma

***Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science & Technology, Hisar, Haryana, Email id: sharmask71@rediffmail.com**

Abstract

Traumatic brain injury (TBI) is being recognized as outstanding cause of mortality and functional disability worldwide. Besides various secondary cascades, a sequence of inflammatory changes takes place after TBI, whereas TNF- α is found to play a deleterious role in acute pathophysiological phase of TBI. TNF- α is being implicated in succession of other inflammatory pathways, further leads to neurological impairments especially in early stages of TBI. The neuroprotection of Kynurenic acid (endogenous metabolite) and its analogues have been established in experimental models of migraine, Huntington's and Alzheimer's disease. In the present study, we evaluated time-dependent neuroprotective effects of Kynurenic acid amide analogue against TNF- α change after traumatic insult in mice. **Methodology:** The current study included swiss albino mice (25-30 g) mice and KAA was synthesized in our laboratory and analyzed using IR and NMR spectroscopy. Timely (6, 24, 48, 72 h and day 21) TNF- α marker and neuroprotection of KAA against it was reported in brain samples after TBI. **Results:** Our data showed dose dependent and time dependent decrease in level of TNF- α after administration of KAA (100, 200, 400 mg/kg, i.p.) after 30 minutes of TBI. **Conclusion:** Our data depicted that KAA induced neuroprotection against TNF- α mediated inflammatory changes and further neurological dysfunctions.

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Key words - TBI, inflammation, TNF- α , Kynurenic acid amide analogue.

Introduction

TBI produces a notable societal and financial burden across the world. The pathology of TBI is complex and multifaceted with assorted pathways engaged in the neuronal dysfunction (Dardiotis et al., 2012). TBI is differentiated into primary and secondary injury. Secondary damage is being treatable phase involved various multiplex devastating features like increase in intracranial pressure, edema, changes in BBB permeability,

oxidative stress, mitochondrial damage, release of excitatory neurotransmitters and cerebral hypoxia (Greve and Zink, 2009). Neuroinflammation is one of secondary cascade happens after TBI. Although, normally central nervous system is an immune privileged organ that restricted the peripheral immune system through BBB (Hickey 1999; Becher et al., 2000). Various evidences suggests that TBI has crucial role in induction of cerebral neuroinflammation including stimulation of resident cells, migration and hiring of leukocytes, release of mediators of inflammation collectively damage the neuronal cells. Cerebral inflammation can be induced by several reasons such as extravasation of blood products, cell debris, intracellular fragments, reactive oxygen and nitrogen species and prostaglandins (Baskaya et al., 1997; Shlosberg et al., 2010). However, TNF- α is one of the central mediator in pathways of neuroinflammation in TBI. TNF- α is 17 kDa pleiotropic peptide, forms multimers and actively interacts with TNF- α receptors (TNFR) exhibited on glia cells and neurons (Merrill, 1991; Wolvers et al., 1993). TNF- α is exhibited a deleterious role in acute Pathophysiology of TBI (Rothwell and Hopkins, 1995; Fan et al., 1996). TNF- α is directly activates inflammation by the stimulation of capillary endothelial cell pro-inflammatory processes, further progression of leukocytes adhesion and infiltration inside the brain (Feuerstein et al., 1994). TNF- α is also being found in blood plasma and cerebrospinal fluid of traumatically injured patients (Ross et al., 1994). The present study investigated the time dependent changes in TNF- α level and neuroprotection by KAA against TNF- α using weight drop model of TBI in mice.

Methodology:

Animals

Swiss albino mice (25-30 g), of either sex were taken for the present study. They were acquired from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India after the approval of the Institutional Animal Ethics Committee (IAEC) of Guru Jambheshwar University of Science & Technology, Hisar, Haryana, India. Animals were kept in groups of five in polypropylene cages (29×22×14 cm) lined with proper bedding. They were conserved under standard conditions (natural light and dark cycle; temperature 25±2°C, Humidity 60-65%) and had free access to standard rodent feed and water *ad libitum*.

Experimental TBI model: A non-penetrating or impact acceleration models of TBI was used in this study where the animal's head has been constrained. Weight drop model utilizes the gravitational forces of free-falling weight on the exposed skull of the animal. Here, the moderate injury was produced in mice with the help of the following parts of model: **Metallic pipe:** (Length: 1 m; Diameter: 2.7 cm); **Metallic disc:** (Thickness: 3 mm; diameter: 1 cm); **Metallic spherical weight** (Weight: 70 g; diameter: 2.5 cm) (Foda and Marmarou, 1994; Marmarou et al., 1994; Chown et al., 2010).

Induction of traumatic brain injury:

Mice were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.), laid down on a sponge pad and a small longitudinal midline incision was marked over the scalp of mice, metallic disc was centrally fixed on the exposed skull and mouse was put appropriately under the metallic pipe. Then the spherical weight was freely fall through the pipe over the skull of mice. Then, the disc was detached and suturing the scalp immediately. Finally, antibacterial (Neosporin) powder (GlaxoSmithKline Pharmaceuticals Ltd., Bangalore, India) was sprinkled over the site of surgery and then mice were returned to their home cage for recovery. Sham group gone through all surgical procedures, except the impact (Marmarou et al., 1994).

Synthesis of Kynurenic acid amide analogue {N-(2-N, N dimethylaminoethyl)-4-oxo-1H quinoline-2-carboxamide hydrochloride:

Amide analogue of Kynurenic acid (N-(2-N, N dimethylaminoethyl)-4-oxo-1H quinoline-2-carboxamide hydrochloride) was prepared in our laboratory using Kynurenic acid as a precursor compound. The reaction was being carried out via amidation process between Kynurenic acid and an amine N, N, dimethylethylene diamine using dimethylformamide as a solvent. 1-hydroxybenzotriazole (Coupling additive) and diisopropylcarbodiimide (Coupling agent) are used for the successful completion of the reaction. The reaction was done at 25°C for 16 hours by stirring with a mechanical stirrer. The reaction mixture was passed through the resin (benzenesulphonic acid) column to separate the by-products and was followed by solvent extraction

in diethyl ether to isolate the analogue. The product was then analyzed by IR and NMR spectroscopy (US Patent Scott, US6362351B2, 26th Mar 2002)

Experimental Plan:

The current study having 07 groups and each group consists of 06 mice.

Group 1: Control; **Group 2:** control + N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide hydrochloride (400 mg/kg, i.p.); **Group 3:** TBI + Vehicle treated (Distilled Water); **Group 4:** Sham (Surgery without TBI); **Group 5:** TBI + N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide hydrochloride (100 mg/kg, i.p.); **Group 6:** TBI + N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide hydrochloride (200 mg/kg, i.p.); **Group 7:** TBI + N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide hydrochloride (400 mg/kg, i.p.)

The N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide hydrochloride were administered by intraperitoneal route after half an hour of injury and repeated after every 24 hours. In present study, estimation of TNF- α were done at 6, 24, 48, 72 h and day 21.

Estimation of TNF- α :

The concentration of TNF- α was measured with the help of mouse tumour necrosis factor ELISA kit (E0117Mo, Bioassay Technology Laboratory). The sensitivity range is 2.52 ng/ml.

Results:

Concentration of TNF- α (6 h):

The level of TNF- α , an inflammatory mediator rises after injury leading to elevation of cerebral neuroinflammation. The concentration of TNF- α was estimated after 6 h of injury. A significant increment ($p < 0.05$) in vehicle treated group was observed after injury when compared to the control and sham group. There was no significant decrement in concentration of TNF- α found in drug treated groups when compared to injured animals.

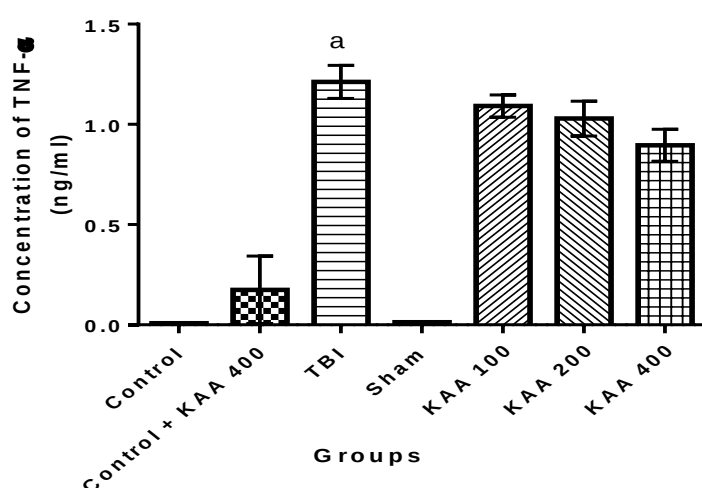


Fig. 1: Effect of Kynurenic acid amide analogue on brain TNF- α at 6 h of injury.

Present study having 7 groups and $n = 6$ in each group. Values are expressed as Mean $\pm$ SEM. Data was analyzed by one way ANOVA followed by a multi-comparison test. **a.** $p < 0.05$ vs control group. The F-value was found to be $F(6, 35) = 39.18$. Control + KAA 400 = *per se* group; KAA 100, 200, 400 stands for N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide 100 mg/kg, 200 mg/kg and 400 mg/kg respectively.

Concentration of TNF- α (24 h):

The concentration of TNF- α was found to be elevated after injury. The level of TNF- α was also observed after 24 h of injury. A significant increment ($p < 0.05$) in vehicle treated group was observed after injury when compared to the control and sham group. There was dose dependent significant ($p < 0.05$) decrement in concentration of TNF- α found in drug treated groups in comparison to injured group

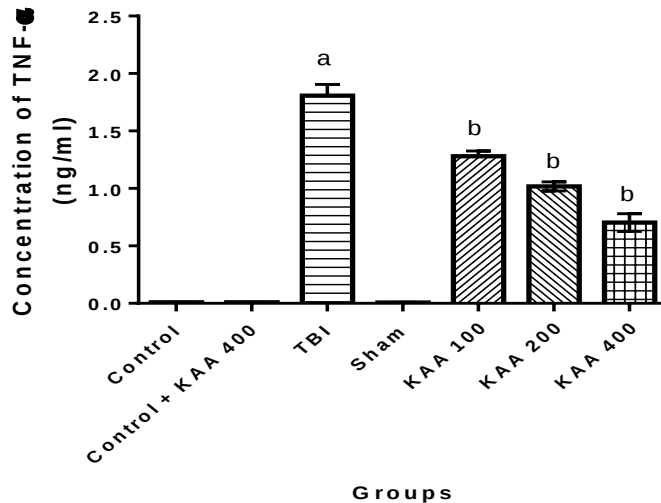


Fig. 2: Effect of Kynurenic acid amide analogue on brain TNF- α at 24 h of injury.

Present study having 7 groups and $n = 6$ in each group. Values are expressed as Mean $\pm$ SEM. Data was analyzed by one way ANOVA followed by a multi-comparison test. **a.** $p < 0.05$ vs control group; **b.** $p < 0.05$ vs TBI group. The F-value was found to be $F(6, 35) = 187.6$. Control + KAA 400 = *per se* group; KAA 100, 200, 400 stands for N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide 100 mg/kg, 200 mg/kg and 400 mg/kg respectively.

Concentration of TNF- α (48 h):

The TNF- α level was also estimated after 48 h of injury. A significant elevation ($p < 0.05$) in injured group was observed after injury when compared to the control and sham group. There was dose dependent significant ($p < 0.05$) decreases in concentration of TNF- α observed in drug treated groups in comparison to TBI group.

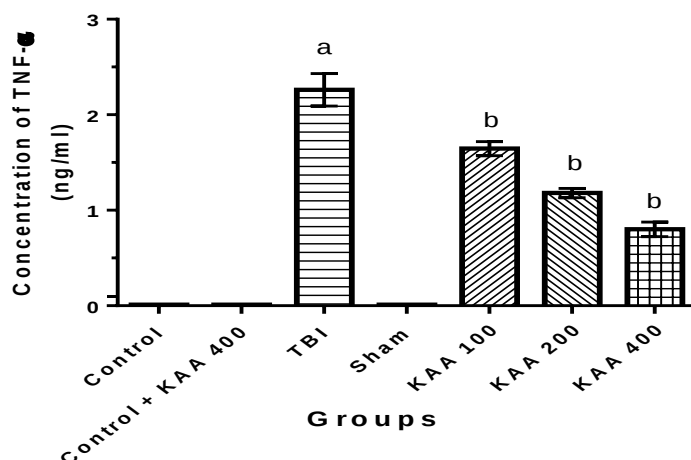


Fig. 3: Effect of Kynurenic acid amide analogue on brain TNF- α at 48 h of injury.

Present study having 7 groups and $n = 6$ in each group. Values are expressed as Mean $\pm$ SEM. Data was analyzed by one way ANOVA followed by a multi-comparison test. **a.** $p < 0.05$ vs control group; **b.** $p < 0.05$ vs TBI group. The F-value was found to be $F(6, 35) = 132.8$. Control + KAA 400 = *per se* group; KAA 100,

200, 400 stands for N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide 100 mg/kg, 200 mg/kg and 400 mg/kg respectively.

Concentration of TNF- α (72 h):

The TNF- α level was also analyzed after 72 h of injury. A significant rise ($p < 0.05$) in inflammation was observed after injury when compared to the control and sham group. There was dose dependent and significant ($p < 0.05$) decrease in concentration of TNF- α was found in drug treated groups in comparison to TBI animals.

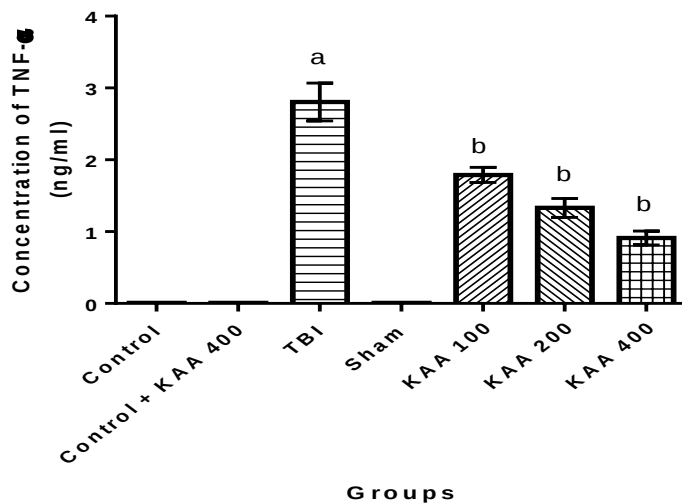


Fig. 4: Effect of Kynurenic acid amide analogue on brain TNF- α at 72 h of injury.

Present study having 7 groups and $n = 6$ in each group. Values are expressed as Mean $\pm$ SEM. Data was analyzed by one way ANOVA followed by a multi-comparison test. **a.** $p < 0.05$ vs control group; **b.** $p < 0.05$ vs TBI group. The F-value was found to be $F(6, 35) = 75.14$. Control + KAA 400 = *per se* group; KAA 100, 200, 400 stands for N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide 100 mg/kg, 200 mg/kg and 400 mg/kg respectively.

Concentration of TNF- α (Day 21):

The TNF- α level was also analyzed after Day 21 of injury. A significant rise ($p < 0.05$) in inflammation was observed after injury when compared to the control and sham group. There was dose dependent and significant ($p < 0.05$) decrease in concentration of TNF- α was found in drug treated groups in comparison to injured mice.

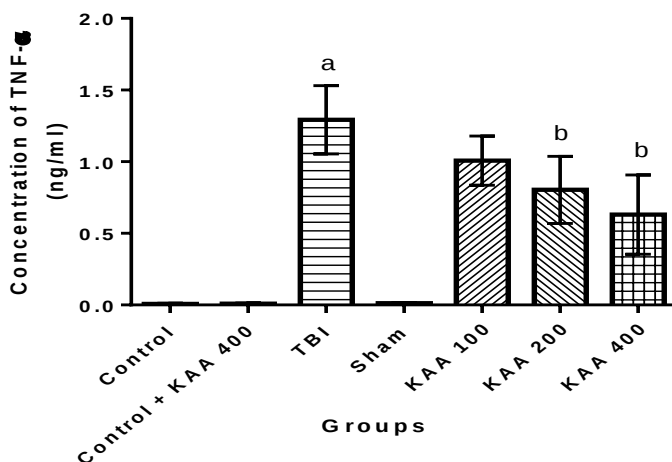


Fig. 5: Effect of Kynurenic acid amide analogue on brain TNF- α at day 21 of injury.

Present study having 7 groups and $n = 6$ in each group. Values are expressed as Mean $\pm$ SEM. Data was analyzed by one way ANOVA followed by a multi-comparison test. **a.** $p < 0.05$ vs control group; **b.** $p < 0.05$ vs TBI group. The F-value was found to be $F(6, 35) = 54.91$. Control + KAA 400 = *per se* group; KAA 100, 200, 400 stands for N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamide 100 mg/kg, 200 mg/kg and 400 mg/kg respectively.

Discussion:

Following a traumatic brain insult, complex and multidimensional secondary cascades ensues (Ghirnikar et al., 1997). Although, inflammation is prime beneficial immune reaction which protects body tissues against harmful substances by halting their survival and multiplication but persistent inflammation plays a deleterious role in functioning of the central nervous system (Allan and Rothwell, 2003; Rothwell, 2003). TNF- α is one of the prime and central mediator which involved in the pathophysiology of TBI because of its upregulation during traumatic insult and various strategies have been developed to suppress the action of TNF- α (Morganti-Kossmann, 1992). In the early phases, TNF- α was found to be secreted by resident cells and then enhances neurological dysfunction in the later phases (Riva-Depaty et al., 1994). Our results was also shown that TNF- α was elevated at early phases i.e. 6h of injury and then progressively increases up to 72 h of injury. The level was also found increased at day 21 but not as much progression like early stages. There was a slight depletion in the later stages might be due to increase in environment conditioning and expression of some neurotrophic factors. Our drug i.e. amide analogue of Kynurenic acid showed a dose dependent (KAA 100, 200, 400 mg/kg, i.p.) significant ($p < 0.05$) decrement in the level of this inflammatory cytokine. However, there was no significant therapeutic action of KAA at early stages of TBI. This depletion might be due to decrement in other devastating secondary cascades such as decrease in brain edema, decrease in BBB permeability, decrease in oxidative stress (Nidhi and Sharma, 2023). The supported studies observed that TNF- α was shown increased expression at early stages in clinical settings also (Goodman et al., 1990; Csuka et al., 1999). The other studies also proved that early upregulation of TNF- α causes neuronal dysfunction (Knoblach et al., 1999). Shohami et al. 1997 observed that inhibition of TNF- α by HU-211, pentoxifyline and other proteins improved neurological outcomes.

Conclusively, TNF- α was shown to be abruptly increased in early stages and a slower progression in later stages which might be deleterious for the brain function. Our drug i.e. KAA showed a good therapeutic potential against increasing concentration of TNF- α . Future studies can further elucidated the other prospective and more detailed analysis of these findings.

References:

1. Allan SM, Rothwell NJ. Inflammation in central nervous system injury. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 2003; 358: 1669-1677.
2. Baskaya MK, Rao AM, Dogan A, Donaldson D, Dempsey RJ. The biphasic opening of the blood-brain barrier in the cortex and hippocampus after traumatic brain injury in rats. *Neurosci Lett* 1997; 226: 33-36.
3. Becher B, Prat A, Antel JP. Brain-immune connection: immuno-regulatory properties of CNS resident cells. *Glia* 2000; 29: 293-304.
4. Csuka E, Morganti-Kossmann MC, Lenzlinger PM, Joller H, Trentz O, Kossmann T. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF- α , TGF- β 1 and blood-brain barrier function. *J Neuroimmunol* 1999; 101: 211-221.
5. Dardiotis E, Karanikas V, Paterakis K, Fountas K, Hadjigeorgiou GM. Traumatic Brain Injury and Inflammation: Emerging Role of Innate and Adaptive Immunity. *Brain Injury – Pathogenesis, Monitoring, Recovery and Management* 2012. Intech publisher, 23-38.
6. Fan L, Young PR, Barone FC, Feuerstein GZ, Smith DH, McIntosh TK. *Mol. Brain Res.* 1996; 36: 287-291.
7. Feuerstein GZ, Liu T, Barone FC. *Cerebrovasc. Brain Metab.* 1994; 6: 341-360.
8. Ghirnikar RS, Lee YL, Engu LF. Inflammation in Traumatic Brain Injury: Role of Cytokines and Chemokines. *Neurochem Res* 1998; 23: 329-340.
9. Goodman JC, Robertson CS, Grossman RG, Narayan RK. Elevation of tumor necrosis factor in head injury. *J Neuroimmunol* 1990; 30: 213-217.
10. Greve MW and Zink BJ. Pathophysiology of traumatic brain injury. *Mt Sinai J Med* 2009; 76: 97-104.

12. Hickey WF. Leukocyte traffic in the central nervous system: the participants and their roles. *Semin Immunol* 1999; 11: 125-137.
13. Khatri N, Sharma S. The Neuroprotective Approach of Amide Analogue of Kynurenic Acid at Mid-Early Stages of Traumatic Brain Injury: A pre-clinical study in mice. *Eur. Chem. Bull.* 2023 12: 13704-13724.
14. Knoblach, SM, SM, Fan L, Faden AI. Early neuronal expression of tumor necrosis factor-alpha after experimental brain injury contributes to neurological impairment. *J Neuroimmunol* 1999; 95: 115-125.
15. Merrill JE. Effects of interleukin-1 and tumor necrosis factor alpha on astrocytes, microglia, oligodendrocytes, and glial precursors in vitro. *Dev Neurosci* 1999; 13: 130-137.
16. Morganti-Kossmann MC, Kossmann T, Wahl SM. Cytokines and neuropathology. *Trends Pharmacol Sci* 1992; 13: 286-291.
17. Riva-Depaty I, Fardeau C, Mariani J, Bouchaud C, Delhaye-Bouchaud N. Contribution of peripheral macrophages and microglia to the cellular reaction after mechanical or neurotoxin-induced lesions of the rat brain. *Exp Neurol* 1994; 128: 77-87.
18. Ross SA, Halliday MI, Campbell GC, Byrnes DP, Rowlands BJ. *Br. J. Neurosurg.* 1994; **8**: 419-425.
19. Rothwell NJ, Hopkins SJ. *Trends Neurosci* 1995; 18: 130-136.
20. Rothwell N. Interleukin-1 and neuronal injury: Mechanisms, modification, and therapeutic potential. *Brain Behav Immun* 2003; 17: 152-157.
21. Shlosberg, D, Benifla M, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat Rev Neurol* 2010; 6: 393-403.
22. Shohami E, Gallily R, Mechoulam R, Bass R, Ben-Hur T. Cytokine production in the brain following closed head injury: dexamethasone (HU-211) is a novel TNF-alpha inhibitor and an effective neuroprotectant. *J Neuroimmunol* 1997; 72: 169-177.
23. Wolvers DA, Marquette C, Berkenbosch F, Haour F. Tumor necrosis factor-alpha: specific binding sites in rodent brain and pituitary gland. *Eur Cytokine Netw* 1993; 4: 377-381.