Effect of $GABA_B$ receptor antagonists (CGP 35348 and CGP 55845) on serum interleukin 6 and 18 concentrations in albino mice following neonatal hypoxia ischemia insult

Quratulane Gillani[1], Muhammad Ali[2] and Furhan Iqbal[1]
[1]Institute of Pure and Applied Biology, Zoology Division, Bahauddin Zakariya University, Multan, Pakistan
[2]Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University Multan, Pakistan

Abstract: Interleukin (IL) 6 and 18 plays an important role in inflammatory response following hypoxia ischemia encephalopathy (HIE). Present study was designed to demonstrate the effect of two $GABA_B$ receptor antagonists (CGP 35348 and 55845), respectively, on the serum IL6 and IL 18 concentrations in albino mice. Albino mice pups (of both genders) were subjected to Murine model of hypoxia-ischemia encephalopathy on postnatal day 10 (right common carotid artery was ligated followed by 8% hypoxia for 25 minutes). After neonatal brain damage and following weaning, mice were divided in three groups, in gender specific manner, and fed on normal rodent diet till they were 13 week old. At this time point, group 1 received intraperitonial saline solution (control group), group 2 was supplemented with CGP 35348 (1mg/ml solvent/Kg body weight) and group 3 with CGP 55845 (1mg/ml solvent/Kg body weight), intraperitonially, for 12 days and IL 6 and 18 concentrations were determined in serum by ELISA. It was observed that CGP 35348 supplementation resulted in reduced interlukin-6 and interlukin-18 concentrations in male albino mice. While CGP 55845 supplementation increased IL-6 and IL-18 concentrations in female albino mice following HIE. Our results are indicating that $GABA_B$ receptor antagonist's supplementation affects IL concentrations in albino mice in a gender specific manner following neonatal brain damage and can be further explored for the treatments of hypoxia ischemia associated neurological ailments.

Keywords: CGP 35348, CGP 55845, Hypoxia Ischemia Encephalopathy; IL6, IL 18, Albino mice.

INTRODUCTION

During gestation or delivery the accidental asphyxia of infants or perinatal hypoxia ischemia (HI) is a general source of neurological deficits (Kaltschmidt *et al.,* 1994). It has been reported that inflammatory mediators, including inflammatory cytokines, add significantly to the pathogenesis that is linked with perinatal brain injury (Bona *et al.,* 1999; Hedtjarn *et al.,* 2002; Eltzschig and Carmeliet, 2011).

In the complex neuro toxic cascade, several inflammatory cytokines interleukin (IL) 6, 1b, 18 and tumor necrosis factor α (TNF-α) are reported to be involved (Jensen, 2001; Plutzky, 2001; Hojo *et al.,* 2002) and as a result a process of self-sustaining secondary neurodegeneration in vulnerable central nervous system (CNS) regions is activated (Northington *et al.,* 2001; Lalouschek *et al.,* 2006). IL-6 has been reported to be up-regulated after focal permanent ischemia in rats (Wang *et al*., 1995) and transient global ischemia in gerbils (Saito *et al*., 1996). IL-18 is a cytokine isolated as an important modulator of immune responses (Gracie *et al.,* 2003). IL-18 and its receptors are expressed in the CNS where they participate in neuro-inflammatory/neurodegenerative processes but also influence homeostasis and behavior (Alboni *et al.,* 2010).

$GABA_B$ receptors are heterodimeric G protein-coupled sites located both pre- and postsynaptic ally in CNS (Stuckey *et al*, 2005). Activation of N-methyl-D-aspartate (NMDA) receptors may induce GABA release, which subsequently inhibits plasmaIL-6 levels through both $GABA_A$ and $GABA_B$ receptors (Song *et al*., 1998). CGP 35348 is a $GABA_B$ receptor antagonist with considerably higher affinity for post versus presynaptic receptors (Olpe *et al.*, 1993b) that crosses the blood–brain barrier (Olpe *et al.,* 1993a). CGP 55845, another recently introduced $GABA_B$ receptor antagonist, was tested on pre- and post-synaptic $GABA_B$ receptors in the hippocampus (Davies *et al.,* 1993) and reported to be more active at presynaptic sites in the hippocampus (Olpe *et al.,* 1994). Present study was designed to demonstrate the effect of $GABA_B$ receptor antagonists (CGP 35348 and CGP 55845) on serum IL-6 and 18 concentrations in albino mice, if any.

MATERIALS AND METHODS

Subject

Albino mice [both male (N=30) and female (N=30)] were used in the present study. Mice were maintained in locally manufactured cages provided with wood chips at the core animal facility in Bio park ofBahauddin Zakariya University, Multan. Albino mice were kept in separate cages with free access to water and standard rodent diet throughout the experimental duration. Room temperature

**Corresponding author:* e-mail: furhan.iqbal@bzu.edu.pk

was maintained at 22±1°C and relative humidity was 50±10%. The light/dark rhythm was 14:10. All the experimental procedures and mouse handling protocols were approved by the ethical committee of Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan.

Murine model of hypoxia-ischemia encephalopathy

On postnatal day 10 of pups, equivalent to the brain growth of 40 weeks gestational age in human fetus (Romijn *et al*., 1991), pups were sedated with isoflurane (3%) inhalation. A right lateral neck cut was made and the right common carotid artery was ligated using polypropylenedalcon USP 6 suture. Pups were kept on a hot plate with stable 36°C temperature during the surgery. The whole surgical procedure was completed within 10 min after which pups were permitted to recover and look after for 30 min with their dams. Mice were then placed for 25 min in a hypoxic chamber with steady flow of 8% Oxygen balanced with Nitrogen. The hypoxic chamber was kept on hot plate to keep the ambient temperature inside the chamber at 36°C. Following hypoxic exposure, pups were taken back to their mothers for revitalization. Following weaning on postnatal day 20, mice were separated from their parents and housed in individual cages.

Experimental Design

Following weaning, mice were separated from their parents and fed on normal mouse diet until 13th week of life when they either received intraperitoneal injections of $GABA_B$ receptor antagonists {CGP 35348 [(3-aminopropyl), (diethoxymethyl) phosphinic acid] (N=5 for each gender) and CGP 55845 [3-N [1-(S)-(3,4-dichlorophenyl) ethyl] amino-2-(S)-hydroxypropyl-P-benzyl-phosphinic acid (N=5 for each gender) at the rate of 1mg/ml solvent/Kg body weight} or saline solution (N=5 for each gender) for 12 days.

Blood sampling

At the end of experiment, blood was sampled from either retro-orbital sinus or through direct cardiac puncture under chloroform inhalation. Blood was centrifuged for ten minutes at 13000 rpm and serum was separated for biochemical analysis of interleukin.

Determination of interleukin 6 and 18

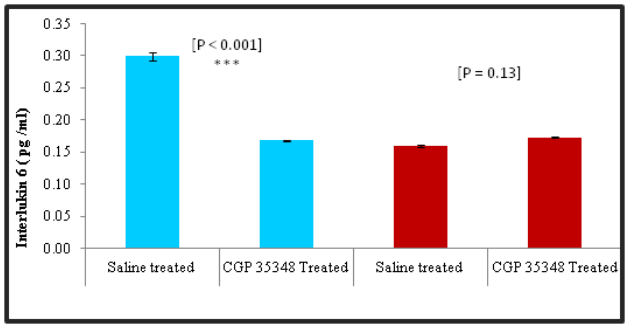
Interlukin 6 was measured by using enzyme-linked immunoabsorbent assay, Mouse 1L-6 antibody pair (Invitrogen, California). Interlukin18 were measured by using enzyme-linked immunoabsorbent assay by using ELISA Kit (MBL, Japan). Ils were determined following the instructions of the kit manufacturers.

STATISTICAL ANALYSIS

All the data is expressed as Mean ± Standard deviation. Statistical package Minitab (version 16, Pennsylvania) was used for the analysis of results. 2 sample t-test was applied to compare IL concentrations between treatment groups following hypoxia ischemia encephalopathy.

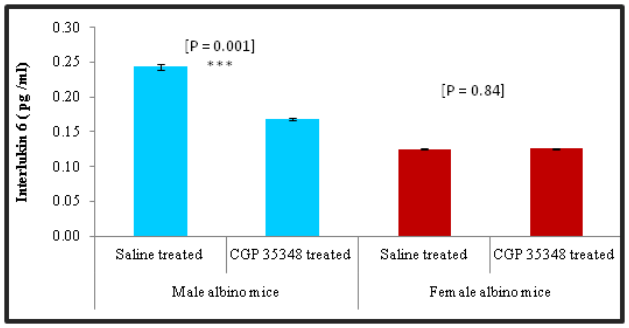
RESULTS

Data analysis revealed that interlukin-6 concentration in serum significantly decreased in male albino mice treated with CGP 35348 in contrast to their saline treated control group following HIE (P<0.001). Female albino mice treated with CGP 35348 had no significant difference in interlukin-6 concentrations as compared to their control group following HIE (fig. 1).



P>0.05=Non significant and P<0.001=Highly significant (***)

Fig. 1: Comparison of Interlukin-6 concentrations in serum between saline and CGP 35348 treated male and female albino mice following hypoxia ischemia encephalopathy. Data is given as mean ± standard error of mean. Two sample t- test results expressed as P - value.

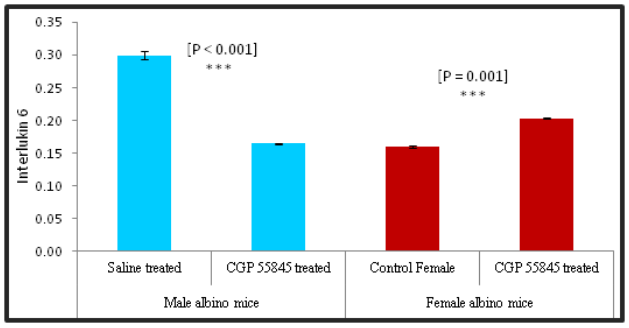


P>0.05=Non significant and P<0.001=Highly significant (***)

Fig. 2: Comparison of Interlukin-18 concentrations in serum of saline and CGP 35348 treated male and female albino mice following hypoxia ischemia encephalopathy. Data is given as mean ± standard error of mean. Two sample t- test results expressed as P - value.

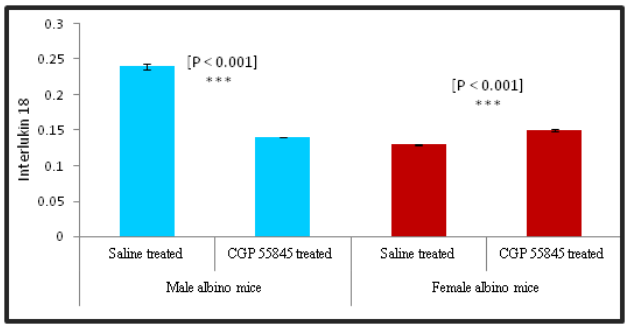
A similar trend was observed when interleukin 18 concentrations in serum were determined. Results revealed that male albino mice treated with CGP 35348 had significantly lower interlukin-18 concentration in serum (P=0.001) when compared to their saline treated control group. CGP 35348 did not affect the levels of interlukin-18 in female albino mice indicating that CGP 35348, in a gender specific manner, affects IL 18 concentration in albino mice following HIE (fig. 2).

When interleukin concentrations were analyzed in CGP 55845 treated and untreated animals, results revealed that interlukin-6 concentration significantly decreased in male albino mice serum when compared to their saline treated control group following HIE (P<0.001). On the other hand female albino mice treated with CGP 55845 had significantly increased interlukin-6 levels (P=0.001) when compared to their saline treated control group following HIE (fig. 3).



P<0.001 = Highly significant (***)

Fig. 3: Comparison of Interlukin-6 concentrations in serum between saline and CGP 55845 treated male and female albino mice following hypoxia ischemia encephalopathy. Data is given as mean ± standard error of mean. Two sample t- test results expressed as P - value.



P<0.001 = Highly significant (***)

Fig. 4: Comparison of Interlukin-18 concentrations in serum of saline and CGP 55845 treated male and female albino mice following hypoxia ischemia encephalopathy. Data is given as mean ± standard error of mean. Two sample t- test results expressed as P - value.

For interlukin-18 concentrations, data analysis revealed that male albino mice treated with CGP 55845 had significantly lower interlukin-18 (P<0.001) concentrations in serum as compared to their saline treated control group. While increased levels of Interlukin-18 (P<0.001) in female albino mice was observed when compared with their saline treated control group following HIE indicating that CGP 55845 also have gender specific effects interleukin concentration in serum (fig. 4).

DISCUSSION

In humans and animal models of hypoxia-ischemia, neuro-inflammation has been extensively studied (Morganti-Kossmann *et al.,* 2007) and overall increase of cytokines in the central nervous system following brain damage has been reported (Stover *et al.,* 2000; Ziebell and Morganti-Kossmann, 2010; Frugier *et al.,* 2010; Xia *et al.,* 2010). Among various cytokines, IL-6 and IL-18 has been extensively studied following brain damage (Del Zoppo*et al.,* 2000; Macrez *et al.,* 2011). IL-6 is produced in response to hypoxia by endothelial cells of peripheral blood vessels (Yan *et al.,* 1997). While in the CNS, ischemia induced IL-6 release from both neurons and microglia (Maeda *et al.,* 1994; Suzuki *et al.,* 1999 a, b). Experimental and clinical studies of different pathological situation including Wallerian degeneration, microbial infections, focal cerebral ischemia, traumatic brain injuries and hypoxic-ischemic suggest that IL-18 is involve in several neuroinflammatory reactions (Felderhoff-Mueser *et al.,* 2005). Recent papers mentioned that thromboembolic stroke cause activation of IL-18 in mice brain (Abulafia *et al.,* 2009) or after hypoxia-ischemia raise of IL-18 levels occurs in young hippocampus of mice (Qiu *et al.,* 2007).

Our results for interlukin-6 and 18 concentrations in serum indicated that CGP 35348 helped in reducing IL-6 and IL-18 levels in male albino mice but they remained unaffected in female albino mice. On the other hand, CGP 55845 reduced the IL-6 and IL-18 concentration in serum in male albino mice but in female albino mice it caused an increased in IL-6 and IL-18 levels in serum following HIE. Continuous increase in the production of plasma IL-6 after brain injury is a sign of poor prognosis and increase of infectious complications (Woiciechowsky *et al.,* 2001; Arand *et al.,* 2001; Kushi *et al.,* 2003). Gopcevic *et al.* (2007) and Berger *et al.* (2009) had reported as increase in IL-6 and IL-18 concentration in serum and cerebrospinal fluid (CSF) of human following moderate and severe traumatic brain injury.

Our results indicated that $GABA_B$ receptor antagonist CGP 35348 and CGP 55845 supplementation was reducing the inflammation in male albino mice only after brain injury. While in female albino mice, CGP 55845 caused an increase in inflammation, which was the sign of poor recovery. An increase in IL-6 levels observed in present studies are supported by several investigations following cerebral ischemia in rodents (Hill *et al.,* 1999; Orzylowska *et al.,* 1999; Suzuki *et al.,* 1999a, b; Ali *et al.,* 2000; Block *et al.,* 2000; Legos *et al.,* 2000). Taupin *et al.,* (1993) had reported an increase in IL-6 expression in both hemispheres after mechanical brain injury in rat brain. Similar observations were reported after transient global ischemia in gerbils (Saito *et al.,* 1996) and focal permanent ischemia in rats (Wang *et al.,* 1995). Song *et*

al., (1998) had reported that injection of baclofen ($GABA_B$ receptor agonist) reduces the plasma IL-6 levels increased by restraint stress. In contrast, injection of 2-hydroxysaclofen ($GABA_B$ receptor antagonist) increased plasma IL-6 levels caused by basal and restraint stress. Effect of baclofen suggested that these $GABA_B$ receptor agonists inhibit central neural circuits, which are involved in the stress-induced, peripheral sympathetic nervous system-mediated increase in plasma IL-6.

In conclusion, we have reported that $GABA_B$ receptor antagonists CGP 35348 helps in reducing the inflammatory interlukin-6 and interlukin-18 concentrations in male albino mice while CGP 55845 causes an increase in IL-6 and IL-18 concentrations in female albino mice following HIE. These findings indicates that neonatal HIE has different mechanisms of brain damage and recovery in albino mice and GABAB receptor antagonist, CGP 35348, may play a role in treatment of hypoxic ischemic brain damage in a gender specific manner but more in detailed studies are required to confirm this observation.

ACKNOWLEDGEMENT

Authors are grateful to Higher Education Commission (HEC) of Pakistan for sponsoring this project through their Indigenous Ph D scheme.

REFERENCES

Abulafia DP, de RiveroVaccari JP, Lozano JD, Lotocki G, Keane RW and Dietrich WD (2009). Inhibition of the inflammasome complex reduces the inflammatory response after thromboembolic stroke in mice. *J. Cereb. Blood. Flow. Metabol.*, **29**: 534-544.

Alboni S, Cervia D, Sugama S and Conti B (2010). Interleukin 18 in the CNS. *J. Neuroinflam.,***7**: 7-9.

Ali C, Nicole O, Docagne F, Lesne S, MacKenzie ET, Nouvelot A, Buisson A and Vivien D (2000). Ischemia induced interleukin-6 as a potential endogenous neuroprotective cytokine against NMDA receptor-mediated excitotoxicity in the brain. *J. Cereb. Blood. Flow. Metabol.*, **20**: 956-66.

Arand M, Melzner H, Kinzl L, Bruckner UB and Gebhard F (2001). Early inflammatory mediator response following isolated traumatic brain injury and other major trauma in humans. *Langenbecks. Arch. Surg.,* **386**: 241.

Berger RP, Ta'asan S, Rand A, Lokshin A and Kochanek P (2009). Multiplex assessment of serum biomarker concentrations in well-appearing children with inflicted traumatic brain injury. *Ped. Res.,* **65**: 97.

Block F, Peters M and Nolden-Koch M (2000). Expression of IL-6 in the ischemic penumbra. *Neuroreport*., **11**: 963-967.

Bona E, Andersson AL, Blomgren K, Gilland E, Puka-Sundvall M, Gustafson K and Hagberg H (1999). Chemokine and inflammatory cell response to hypoxia-ischemia in immature rats. *Ped. Res.,* **45**: 500-509.

Davies CH, Pozza MF and Collingridge GL (1993). CGP 55845A: A potent antagonist of $GABA_B$ receptors in the CA1 region of rat hippocampus. *Neuropharma.,* **32**(10): 1071-1073.

Del Zoppo G, Hallenbeck JM, Iadecola C, Wang XK and Feuerstein GZ (2000). Inflammation and stroke: Putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. *Brain. Pathol.,* **10**: 95-112.

Eltzschig KH, Carmeliet P (2011). Hypoxia and inflammation. *N. Eng. J. Med.,* **364**(7): 656-665.

Felderhoff-Mueser U, Schmidt OI, Oberholzer A, Buhrer C and Stahel PF (2005). IL-18: a key player in neuroinflammation and neurodegeneration? *Trend. Neurosci.,* **28**: 487-493.

Frugier T, Morganti-Kossmann MC, O'Reilly D and McLean CA (2010). In situ detection of inflammatory mediators in post mortem human brain tissue after traumatic injury. *J. Neurotrauma.,* **27**: 497-507.

Gopcevic A, Mazul-Sunko B and Marout J (2007). Plasma interleukin-8 as a potential predictor of mortality in adult patients with severe traumatic brain injury. *Tohoku. J. Exp. Med.,* **211**: 387.

Gracie JA, Robertson SE, McInnes IB (2003). Interleukin-18. *J. Leukoc. Biol.,* **73**(2): 213-224.

Hedtjarn M, Leverin AL, Eriksson K, Blomgren K, Mallard C and Hagberg H (2002). Interleukin-18 involvement in hypoxic-ischemic brain injury. *J. Neurosci.,* **22**: 5910-5919.

Hill JK, Gunion-Rinker L, Kulhanek D, Lessov N, Kim S, Clark WM, Dixon MP, Nishi R, Stenzel-Poore MP and Eckenstein FP (1999). Temporal modulation of cytokine expression following focal cerebral ischemia in mice. *Brain. Res.,* **820**: 45-54.

Hojo Y, Ikeda U, Takahashi M and Shimada K (2002). Increased levels of monocyte-related cytokines in patients with unstable angina. *Atheroscler.,* **161**: 403-408.

Jensen FE (2001). Developmental factors regulating susceptibility to perinatal brain injury and seizures. *Curr. Opin. Pedi.,* **8**: 628-633.

Kaltschmidt C, Kaltschmidt B, Neumann H, Wekerle H, Baeuerle PA (1994). Constitutive NF-kappa B activity in neurons. *Mol. Cell. Bio.,* **14**: 3981-3992.

Kushi H, Saito T, Makino K and Hayashi N (2003). IL-8 is a key mediator of neuroinflammation in severe traumatic brain injuries. *Acta. Neurochir. Suppl.,* **86**: 347.

Lalouschek W, Schillinger M, Hsieh K, Endler G, Greisenegger S, Marculescu R, Lang W, Wagner O, Cheng S and Mannhalter C (2006). Polymorphisms of the inflammatory system and risk of ischemic

cerebrovascular events. *Clin. Chem. Lab. Med.,* **44**: 918-923.

Legos JJ, Whitmore RG, Erhardt JA, Parsons AA, Tuma RF and Barone FC (2000). Quantitative changes in interleukin proteins following focal stroke in the rat. *Neurosci. Lett.,* **282**: 189-192.

Loddick SA, Turnbull AV and Rothwell NJ (1998). Cerebral Interlukin-6 is neuroprotective during permanent focal cerebral ischemia in the rat. *J. Cere. Blo. Flow. Meta.,***18**: 176-179.

Macrez R, Ali C, Toutirais O, Le Mauff B, Defer G, Dirnagl U and Vivien D (2011). Stroke and the immune system: From patho physiology to new therapeutic strategies. *Lancet. Neurol.,* **10**: 471-480.

Maeda Y, Matsumoto M, Hori O, Kuwabara K, Ogawa S, Yan SD, Ohtsuki T, Kinoshita T, Kamada T, Stern DM (1994). Hypoxiareoxygenation- mediated induction of astrocyte interleukin-6: A paracrine mechanism potentially enhancing survival. *J. Exp. Med.,* **180**: 2297-2308.

Morganti-Kossmann MC, Satgunaseelan L, Bye N and Kossmann T (2007). Modulation of immuneresponse to head injury. *Injury,* **38**: 1392.

Naldini A, Carraro F, Silvestri S and Bocci V (1997). Hypoxia affects cytokine production and proliferative responses by human peripheral mononuclear cells. *J. Cell. Physiol.,* **173**: 335-342.

Northington FJ, Ferriero DM, Flock DL and Marin LJ (2001). Delayed neurodegeneration in neonatal rat thalamus after hypoxia-ischemia is apoptosis. *J. Neurosci.,* **21**: 1931-1938.

Olpe H-R, Steinmann MW, Greiner K and Pozza MF (1994). Contribution of presynaptic GABA-B receptors to paired-pulse depression of GABA responses in the hippocampus. *Arch. Pharmacol.,* **349**: 473-477.

Olpe HR, Steinmann MW, Ferrat T, Pozza MF, Greiner K, Brugger F, Froestl W, Mickel SJ and Bittiger H (1993a). The actions of orally active GABAB receptor antagonists on GABAergic transmission *in vivo* and *in vitro*. *Eur. J. Pharmacol.,* **233**: 179-186.

Olpe HR, Wo¨rner W and Ferrat T (1993b). Stimulation parameters determine role of GABAB receptors in long-term potentiation. *Experientia.,* **49**: 542-546.

Orzylowska O, Oderfeld-Nowak B, Zaremba M, Januszewski S and Mossakowski M (1999).Prolonged and concomitant induction of astroglial-immunoreactivity of interleukin-1beta and interleukin-6 in the rat hippocampus after transient global ischemia. *Neurosci. Lett.,* **263**: 72-76.

Plutzky J (2001). Inflammatory pathways in atherosclerosis and acute coronary syndromes. *Am. J. Cardiol.,* **88**: 10K-15K.

Qiu L, Zhu C, Wang X, Xu F, Eriksson PS, Nilsson M, Cooper-Kuhn CM, Kuhn HG and Blomgren K (2007). Less neurogenesis and inflammation in the immature than in the juvenile brain after cerebral hypoxia-ischemia. *J. Cereb. Blood. Flow. Metabol.,* **27**: 785-794.

Romijn HJ, Hofman MA and Gramsbergen A (1991). At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby? *Early. Hum. Dev.,* **26**: 61-67.

Saito K, Suyama K, Nishida K, Sei Y, Basile AS (1996). Early increases in TNF-a, IL-6 and IL-l 13 levels following transient cerebral ischemia in gerbil brain. *Neurosci. Lett.,* **206**: 149-152.

Smith CJ, Emsley HCA, Gavin CM, Georgious RF, Vail A, Barberan EM, Zoppo GJD, Hallenbeck JM, Rothwell N, Hopkins SJ and Tyrrell P (2004). Peak plasma interlukin-6 and other peripheral markers of inflammation in the first week of ischemia stroke correlate with brain infract volume, stroke severity and long term outcome. *BMC. Neuro.,* 4.

Song DK, Suh HW, Huh SO, Jung JS, Ihn BM, Choi IG and Kim YH (1998). Central GABA A and GABA B receptor modulation of basal and stress induced plasma Interlukin-6 levels in mice. *J. Pharm. Exper. Theru.,* **287**(1): 144-149.

Stover JF, Beyer TF, Woiciechowsky C and Unterberg AW (2000). Temporal profile of cerebrospinal fluid glutamate, interleukin-6 and tumor necrosis factoralpha in relation to brain edema and contusion following controlled cortical impact in rats. *Neurosci. Lett.,* **288**: 25-28.

Stuckey DJ, Anthony DC, Lowe JP, Miller J, Palm WM, Styles P, Perry VH, Blamire AM and Sibson NR (2005). Detection of the inhibitory neurotransmitter GABA in macrophages by magnetic resonance spectroscopy. *J. Leukoc. Biol.,* **78**: 393-400.

Suzuki S, Tanaka K, Nagata E, Ito D, Dembo T and Fukuuchi Y (1999a). Cerebral neurons express interleukin-6 after transient forebrain ischemia in gerbils. *Neurosci. Lett.,* **262**: 117-120.

Suzuki S, Tanaka K, Nogawa S, Nagata E, Ito D, Dembo T and Fukuuchi Y (1999b). Temporal profile and cellular localization of interleukin-6 protein after focal cerebral ischemia in rats. *J. Cereb. Blood. Flow. Metab.*, **19**: 1256-1262.

Taupin V, Toulmond S, Serrano A, Benavides J, Zavala F (1993). Increase in lL-6, IL-l and TNF levels in rat brain following traumatic lesion. *J. Neuroimmuno.,* **42**: 177-186.

Wang X, Yue TL, Young PR, Barone FC and Feuerstein GZ (1995). Expression of interleukin-6, c-fos and zif268 mRNAs in rat ischemic cortex. *J. Cereb. Blood. Flow. Metab.,* **15**: 166-171.

Woiciechowsky C, Schoning B, Cobanov J, Lanksch WR, Volk HD and Docke WD (2001). Early IL-6 plasma concentrations correlate with severity of brain injury and pneumonia in brain-injured patients. *J. Trauma.,* **52**: 339.

Xia W, Han J, Huang G and Ying W (2010). Inflammation in ischaemic brain injury: Current advances and future

perspectives. *Clin. Exp. Pharmacol. Physiol.,* **37**: 253-258.

Yan SF, Ogawa S, Stern DM and Pinsky DJ (1997). Hypoxia-induced modulation of endothelial cell properties: Regulation of barrier function and expression of interleukin-6. *Kidney. Int.,* **51**: 419-425.

Ziebell JM and Morganti-Kossmann MC (2010). Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. *Neurotherap.,***7**: 22-30.