Review Article

Indian J Med Res 133, January 2011, pp 15-26

Potential of immunosuppressive agents in cerebral ischaemia

Yogendra Kumar Gupta & Anjali Chauhan

Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India

Received July 27, 2009

Ischaemic stroke is a disorder involving multiple mechanisms of injury progression including activation of glutamate receptors, release of proinflammatory cytokines, nitric oxide (NO), free oxygen radicals and proteases. Presently, recombinant tissue plasminogen activator (rtPA) is the only drug approved for the management of acute ischaemic stroke. This drug, however, is associated with limitations like narrow therapeutic window and increased risk of intracranial haemorrhage. A large number of therapeutic agents have been tested including N-methly-D-aspartate (NMDA) receptor antagonist, calcium channel blockers and antioxidants for management of stroke, but none has provided significant neuroprotection in clinical trials. Therefore, searching for other potentially effective drugs for ischaemic stroke management becomes important. Immunosuppressive agents with their wide array of mechanisms have potential as neuroprotectants. Corticosteroids, immunophilin ligands, mycophenolate mofetil and minocycline have shown protective effect on neurons by their direct actions or attenuating toxic effects of mediators of inflammation. This review focuses on the current status of corticosteroids, cyclosporine A, FK506, rapamycin, mycophenolate mofetil and minocycline in the experimental models of cerebral ischaemia.

Key words Cerebral ischaemia - immunosuppressants - *in vitro* and *in vivo* models - neuroprotection

Introduction

Cerebral stroke (brain attack) is the most life-threatening cerebovascular disorder, the second leading cause of death and principle cause of disability in the world[1]. Even with advances in treatment of stroke, 20-50 per cent of the patient die within a month or become dependent on others[2]. Stroke results due to interruption of cerebral blood flow causing irreversible and fatal damage to the affected neurons. There are two main types of strokes, ischaemic and haemorrhagic. Ischaemic stroke accounts nearly for 85 per cent of all reported stroke incidents and is the main focus of the current studies. This type of stroke occurs when a thrombus or embolus blocks cerebral blood flow resulting in cerebral ischaemia and consequently neuronal damage and cell death. Haemorrhagic stroke occurs due to rupture of any blood vessel in the brain resulting in rapid cerebral damage and accounts for the remaining 15 per cent stroke cases.

Intravenous recombinant tissue plasminogen activator (rtPA) is the only approved therapy for management of ischaemic stroke[3]. Patients who receive this drug within the initial 3 h therapeutic window also have a high risk of intracranial haemorrhage, usually 6-8 per cent against 0.6-2 per cent spontaneous hemorrhages in stroke[4-5]. Other limitations associated with rtPA therapy like disruption of blood brain barrier; seizures and progression of neuronal damage[6-8] are

major concerns. Thus, there is a continued need for exploring novel neuroprotective strategies for the management of ischaemic stroke.

Recent studies on immunosuppressive agents have revealed their neuroprotective potential in ischaemic stroke. Immunosuppressive agents have shown promise as being neuroprotective in safeguarding the neurons against excitotoxic insults and also improving neurological functions and infarct volume in experimental models of ischaemic stroke[9-13]. These agents have direct effect on microglia cells and inhibit mediators of inflammation. In order to appreciate the potential role of immunosuppressive agents in ischaemia, revisiting the pathophysiology of cerebral ischaemia is required. This review briefly focuses on the mechanisms involved in cerebral ischaemic stroke and how the immunosuppressive agents have shown potential in its management.

The aetiopathology and mechanisms of cell death in ischaemia

The interruption in blood flow to the brain results in reduced supply of oxygen and nutrients to the neurons. The lack of blood supply results in two identifiable areas namely the core and penumbra. The core which is a neuronal dead area is not accessible to therapeutic intervention whereas the penumbra is a still salvageable zone and is the target of the most therapeutic interventions (Fig.). The consequence of ischaemia can briefly be described as below.

Energy depletion: Consequent to reduction/loss of blood supply inside the core, the adenosine triphosphate (ATP) levels are reduced leading to incarceration of cellular metabolism[14]. The lack of energy results in impaired ion homeostasis.

Calcium overload and activation of glutamate receptors: Disrupted ion homeostasis leads to rapid depolarization, and large influx of calcium and potassium. The intracellular calcium overload results in activation of excitotoxic glutamatergic transmission, nitric oxide (NO) synthase, caspase, xanthine oxidase and release of reactive oxygen species[15] (Fig.).

Excess glutamate release leads to activation of phospholipases, phospholipid hydrolysis and arachidonic acid release, ultimately resulting in necrotic as well as apoptotic cell death[16-18]. Generation of free radicals, lipid peroxidation, inflammatory cascade and activation of immediate early genes such as *c-fos*, *c-jun*, leads to progressive ischaemic damage.

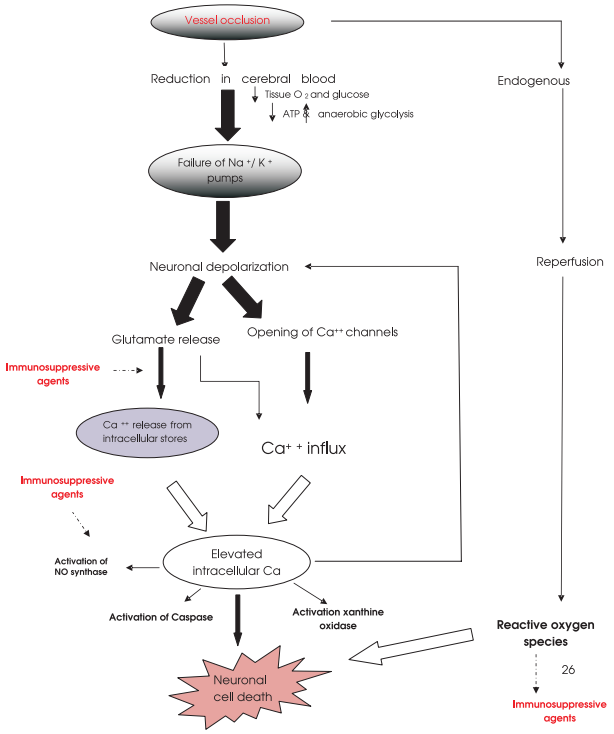

Fig. A simplistic presentation of the cascade of events occurring in cerebral ischaemia and possible sites of immunosuppressive agents actions.

The generation of reactive oxygen species in the core area is less as compared to penumbra because there is negligible blood supply to core. The penumbra is still the viable tissue surrounding the core, and receives trivial amount of blood from collateral arteries[19]. This area is therefore, the target for drug intervention and has potential for recovery. If no quick and effective drug intervention is done, this further progresses to cellular energy failure, release of excitotoxic neurotransmitters and reactive oxygen species and finally, cellular death in the region.

Mechanism of cell death

Neurodegeneration

Neurodegeneration is described by the loss of neuronal functions and cell death[20]. Neuronal degeneration and cell death occurs through apoptotic as well as necrotic pathways[21]. Cell death is characterized by imbalance in cellular homeostasis, influx of calcium, mitochondrial dysfunction and generation of reactive free radicals[22,23].

(*i*) Necrotic cell death - Energy depletion leads to release of excitatory amino acids (EAAs) such as

glutamate[24]. Excessive release of glutamate activates α-amino-3-hydroxy-5-methlyl-4-isoxazolone-proprionic acid (AMPA) receptors which increase sodium influx[25] and the N-methly-D-aspartate (NMDA) receptors which mediate the influx of calcium[26]. This increase in calcium overload results in activation of proteases such as caspases and matrix metalloproteases (MMP). The aftermath of this activation is increased proteolytic injury and ultimately cell death[27]. Many calcium dependent and calcium induced enzymes mediate intracellular calcium induced toxicity like NO synthase, cyclooxygenase, phospholipase A_2 and calpain1[28]. Increased intracellular calcium activates NO synthase and results in release of NO[29]. The NO then combines with superoxide generated as by-product from cyclooxygenase, or xanthine oxidase to produce highly reactive peroxynitrite, that results in tissue destruction[30]. Cells are not capable of protecting themselves against excessive reactive oxygen species and ultimately die.

(*ii*) Apoptotic cell death - In apoptotic cell death there is transcription of immediate early genes such as *c-fos*, *c-jun* leading to caspase cascade resulting in increased cytokine levels within hours of initial injury. The released cytokines cause activation of cell surface receptors such as Fas receptor and tumour necrosis factor-alpha receptor (TNF- α) leading to apoptotic cell death[31,32]. TNF- α stimulates the production of bcl-2 family protein, bid[33]. Bid activates bax, another bcl-2 family member and increases mitochondrial permeability, resulting in release of cytochrome c, a key component in apoptosis initiation. Cytochrome c forms a complex with apoptotic protease activating factor-1 (APAF -1) and procaspase -9, this complex causes cleavage of procaspase – 9 to caspase 9 and ultimately activation of other caspases including casapase-3[34]. Caspase-3 injury leads to irreversible DNA damage and cell death[35]. Generation of reactive oxygen species during cerebral ischaemia also activates process of apoptosis[36] leading to activation of transcription factor p53 and caspases thus resulting in DNA damage[37].

Inflammation and ischaemia - role of microglia cells

The continued ischaemic injury to brain cells results in to a complex inflammatory cascade. It is characterized by infiltration of leukocytes mainly polymorphonuclear (PMN) cells, monocytes/macrophages lymphocytes and the activation of microglia which are the resident immune cells of the brain[38]. Astrocytes, neuronal support cells, also contribute to inflammation during insult[39]. Astrocytes under normal conditions perform several functions like, glutamate uptake, glutamate release and maintain cellular and ion homeostasis[40]. During cerebral injury, astrocytes undergo morphological changes and become activated[41]. Activated astrocytes release proinflammtory cytokines and chemokines thus results in initiation and progression of inflammation[39].

In cerebral ischaemia, microglia, resident brain macrophages become activated and release detrimental neurotoxic mediators like proinflammtory cytokines, superoxide, nitric oxide (NO), TNF- α and proteases[42-44]. Many of these mediators can inturn influence the microglia morphology and activate it in a paracrine and autocrine fashion[45]. Among the proinflammatory cytokines interleukin- 1 (IL-1) is most abundantly expressed in microglia cells[46]. IL-1 induces the expression of other cytokines such as IL-6 and TNF-α[47] which contribute to the progression of ischaemic damage. The inhibition of this pathway of neuronal damage can be considered a logical option.

Other agents that have an important role in stroke injury are proteases. Tissue plasminogen activator, a potential neurotoxicant released by microglia cells is implicated in cerebral ischaemic injury[48]. Other proteases that play a leading role in disruption of blood brain barrier are matrix metalloproteases which contribute to secondary brain damage in cerebral ischaemia[49]. NO is a small molecule, secreted by microglia cells, acts directly on neurons as neurotoxin or indirectly by potentiating excitotoxic transmitters[50]. Inhibition of microglia activation in ischaemia may provide a novel target in management of stroke, the immunosuppressive agents, therefore, may serve the purpose to a greater extent.

Is rtPA good enough in acute stroke?

Currently, recombinant tissue plasminogen activator (rtPA) is the only drug approved by US Food and Drug Administration for management of acute ischaemic stroke[51]. Several limitations are associated with rtPA including the narrow 3 h therapeutic window, increased risk of intracranial haemorrhage, generation of free oxygen radicals and recurrent stroke[5,51]. The rtPA has only clot busting activity and this activity is bothersome because it can lead to progression of secondary neuronal damage[52].

For developing potent neuroprotective agents two main strategies are focused, clot lysis activity and safeguard of neurons subjected to ischaemic damage. rtPA is adequate for clot lysis, but there have been

many unsuccessful attempts in developing effective neuroprotective drugs for management of stroke.

Drugs under investigation for ischaemic stroke

Several studies conducted on drugs of different groups have exhibited neuroprotection in experimental models of cerebral ischaemia. One of these agents is endothelin antagonist (TAK-044), a reported anti-inflammmatory and antioxidant agent[53]. In oxygen-glucose deprivation model of cerebral ischaemia, TAK-044 showed significant improvement in percentage cell viability as compared to cells in hypoxic condition[54]. In rat model of focal cerebral ischaemia, TAK-044 significantly improved the neurological parameters, oxidative stress markers and infarct volume[53]. Several other agents have also shown potential of protecting the ischaemic cerebral injury by antioxidant mechanism. Agents like α–tocopherol[55], trans–resveratrol[56] and melatonin[57] have shown significant improvement in neurobehavioural paradigms, infarct size and oxidative stress markers in experimental model of cerebral ischaemia.

The other group of drugs that showed promise is statins, 3-hydroxy-3-methyglutaryl (3- HMG)- CoA reductase inhibitors. Several studies have reported the beneficial effects of statins in experimental model of cerebral ischaemia[58-60]. The neuroprotective effects of statins are reported to be mediated by inhibiting inducible NO synthase thus resulting in inhibition of proinflammtory cytokines[60]. Beside these agents, recent studies have also shown neuroprotection by human umbilical cord cells[61, 62] in experimental models of cerebral ischaemia.

Recent findings have focused on drugs which can be given as stand alone or in combination with rtPA for management of stroke. One of these agents is imatinib, an anticancer drug. Imatinib is tyrosine kinase inhibitor used in treatment of chronic myeloid leukaemia. The rtPA neurotoxicity is thought to be mediated through platelet derived growth factor–CC (PDGF-CC)[63]. Recently Su *et al*[63] have shown that administration of imatinib, PDGF-CC antagonist, one hour after vessel occlusion and 5 h later application of rtPA drastically reduced the infarct volume by 34 per cent and incidence of intracranial haemorrhage by 50 per cent as compared to rtPA alone in mouse model of stroke. One single administration of imatinib before the rtPA administration may provide an adequate neuroprotection in stroke patients, but this aspect needs to be validated in clinical settings. Till these agents are tested in clinical trials, no concrete conclusions can be drawn from animal models.

Neuroprotection by immunosuppressive agents: a new dimension

As the ischaemic stroke has a multifactorial aetiopathology, and the mechanisms that are involved are activation of glutamate receptors, release of proinflammatory cytokines, TNF-α, NO, reactive oxygen radicals and proteases, the drugs having combinational neuroprotective and anti-inflammatory activity will be theoretically more effective in its management. Immunosuppressive agents have shown potential as neuroprotectants and anti-inflammatory agents in experimental models of cerebral ischaemia (Fig.). Drugs including steroids, immunophilin ligands and mycophenolate mofetil (MMF) have been in use as immunosuppressants since 1970s and these have been effectively used in avoiding organ rejection in transplant patients. Recent studies have reported their protective effect in microglial and neuronal cell cultures and in animal models of cerebral ischaemia[64]. Steroids, cyclosporine A, FK506, MMF and minocycline have been reported to have direct inhibitory effects on activation of microglia cells. This effect is mediated by inhibition of secretion of proinflammtory cytokines and NO from microglia cells[64] (Table).

Steroids in cerebral ischaemia: an unresolved issue

For many years corticosteroids have been used for the treatment of brain oedema[65]. Overwhelming evidence has reported the protective effects of corticosteroids, both *in vitro* as well as in a variety of animal models[66,67].

In vitro studies using steroids have shown inhibition of both microglial cell proliferation and activity of NO synthase[68] in the isolated microglial cells. Several investigators have reported that treatment of isolated microglial cells with steroid reduce the release of the proinflammatory substances[69-71] and thus restricting the inflammation cascade and protecting neurons against the ischaemic insult. Treatment with dexamethasone on gerbil hippocampal regions diminished the ischaemia induced glutamate toxicity and consequently protected the neurons[10]. Dexamethasone has also been shown to suppress monocyte chemoattractant protein-1 (MCP-1) production via inhibition of jun-N-terminal kinase and p38 mitogen activated protein kinase in activated microglial cells[8].

Table. Effects produced by immunosuppressive agents in experimental models of ischaemic stroke

Immunosuppressive drugs investigated	Experimental model	Effect produced	References
Steroids (Cortisol, corticosterone & dexamethasone)	Isolated glial cells	Inhibition proliferation and activity of iNOS. Inhibition of release of TNF- α and IL-6. Suppression of production of MCP-1.	Chao *et al*, 1992[69]; Drew & Chavis, 2000[70]; Jacobsson *et al*, 2006[71]
Dexamethasone	Permanent middle cerebral artery occlusion model in rats.	Reduction in expression of TNF- α.	Bertorelli *et al*, 1998[72]
	Permanent middle cerebral artery occlusion model in cats.	Beneficial effect on cerebral oedema.	Fenske *et al*, 1979[73]
	Occlusion of middle cerebral arteries in hyperglycaemic cat.	Reduce size of infarcts.	de Courten-Myers, 1994[74]
Cyclosporine A	Rat neuronal cells.	Inhibit caspase activation.	Capano *et al*, 2002[9]
	Human neuroblastoma cells.	Prevents apoptosis and enhance neurite outgrowth.	Sheehan *et al*, 2006[84]
	Transient forebrain ischaemia in rats.	Reduced brain oedema and infarct size.	Shiga *et al*, 1992[85]
	Hippocampal CA1 neurons.	Improvement in survival.	Uchino *et al*, 1995[86]; Miyata *et al*, 2001[87]
	Global ischaemia rat model.	Inhibit activation of microglia cells secreted proinflammatory substances.	Wakita *et al*, 1995[88]
Tacrolimus, FK506	Cortical cell against excitotoxic neuronal death.	Prevents dephosphorylation of nitric oxide synthase.	Dawson *et al*, 1993[92]
	Rodent model of focal ischaemia.	Neuroprotective effect.	Sharkey and Butcher, 1994[11]
	Transient global ischaemia model in gerbils.	Reduce delayed neuronal death mediated by inhibition of neuronal nitric oxide synthase.	Tokime *et al*, 1996[94]
	Primate model of cerebral ischaemia.	Improved neurological functions.	Furuichi *et al*, 2006[95]
Rapamycin	Microglial cells.	Inhibits expression of inducible nitric oxide synthase.	Lu *et al*, 2006[100]
	Traumatic injury model in mice.	Improved the functional recovery.	Erlich *et al*, 2007[101]
Mycophenolate Mofetil	Organotypic hippocampal slice cultures.	Attenuated neuronal damage induced by excitotoxic injury.	Dehghani *et al*, 2003[104]
	Mice model of amyotrophic lateral sclerosis.	Reduced microglial activation, improved stem cell survival and delayed the onset of neurological symptoms.	Yan *et al*, 2006[106]
Minocycline	Mixed SC and pure microglia cultures.	Inhibition of NMDA activated p38 MAPK, No and IL- 1β release.	Tikka *et al*, 2001[112]
	Global ischaemia in gerbils.	Inhibition of microglial cells.	Yrjanheikki *et al*, 1998[12]
	Focal ischaemia in rats.	Reduced infarct volume & Inhibition of microglial cells.	Yrjanheikki *et al*, 1999[13]

iNOS, inducible nitric oxide synthase; TNF- α, tumor necrosis factor-alpha; IL-6, interleukin- 1; MCP-1, monocyte chemoattractant protein-1; NMDA, *N*-methyl-D-aspartate; p38 MAPK, p38 mitogen-activated protein kinase; NO, nitric oxide

In *in vivo* studies, application of dexamethasone in permanent middle cerebral artery occlusion model in rats reduced the expression of TNF-α and decreased the inflammatory cascade[72]. Dexamethasone treatment in a model of permanent occlusion of middle cerebral artery in cats showed neuroprotective effects[73]. In an interesting experiment by de Courten – Myers *et al*[74], 4 h occlusion of middle cerebral arteries in hyperglycaemic cats, and administration of high dose of corticosteroids 30 min after occlusion, significantly reduced the size of infarcts as compared to untreated group.

The mechanism of neuroprotection by corticosteroids is speculated to be either due to activation of membrane receptors on neurons by circulating steroids and hence leading to rapid changes in properties of exposed neuron or their modulation of nuclear transcription of various genes[67]. The beneficial effects of corticosteroids are largely due to preservation of neuronal structures and improvement in behavioural functions.

Interestingly in contrast to the experimental evidences, in the clinical trials, the steroids have not shown any beneficial effects. In a retrospective study on patients with history of ischaemic stroke, disability and mortality rates were worse in patients taking steroids as compared to those without steroid treatment[75]. There was no difference between the outcomes *i.e*., the disability and mortality as well as adverse effects between the two groups. In another study, patients with acute cerebral infarction were treated either with dexamethasone or placebo and the patients in steroid group fared worse than the placebo group[76].

In summary, corticosteroids have shown favourable neuroprotective profile in experimental models of ischaemic stroke but failed to provide any significant beneficial effect in ischaemic patients. Thus, the use of corticosteroids as neuroprotective agents still remains controversial and there is a need to re-evaluate these in well planned clinical trials.

How effective are immunophilin ligands in stroke?

A plethora of evidence is available that indicates the role of immunophilin/calcineurin in brain function and development[77-79]. High levels of immunophilins are expressed in brain and these are believed to be involved in neuronal apoptosis mechanisms[80]. Cyclosporin, tacrolimus/FK506 and rapamycin are immunophilin binding ligands used in avoiding rejection of transplanted organs.

Cyclosporine A: Cyclosporine A is an 11 amino acid cyclic peptide of fungal origin having effective immunosuppressive action. The mechanism of action of cyclosporine A involves binding to cyclophilin, an intracellular protein belonging to immunophillin family. Cyclosporine A and cyclophillin complex inhibits calcium/calmodulin dependent activation of calcineurin and via this pathway cyclosporine inhibits production of IL-2[81] and gamma interferon and other lymphokines[82]. Cyclosporine A can also reduce the expression of an intracellular adhesion molecule and affect subsequent inflammation cascade[83].

Cyclosporine A has shown antiapoptotic activity by inhibiting activation of caspase in rat neuronal cells[9]. It showed protective effect against apoptosis in human neuroblastoma cells by enhancing neurite outgrowth[84]. Cyclosporine A has shown to reduce brain oedema, infarct size and improved the survival of hippocampal CA1 neurons probably due to enhanced phosphorylation of cAMP response element binding (CREB) protein and increase in production of brain derived neurotrophic factor in experimental model of transient forebrain ischaemia in rats[85-87]. In the global ischaemia rat model, cyclosporine A showed beneficial effects by inhibiting the activation of microglia cells and thus providing protection against microglia secreted proinflammatory substances[88]. Till now cyclosporine A has not been tested in clinical trials for the treatment of CNS disorders but it has been tried in autoimmune disorders.

Cyclosporine A treatment in rats with experimental autoimmune myasthenia gravis has shown protective effects by reducing the levels of acetylcholine receptor antibodies by 50 per cent[89]. Mahattanakul *et al*[90] have reported antinflammatory effect of cyclosporine A in patients suffering from chronic inflammatory demyelinating polyneuropathy. In this study, cyclosporine A treatment was successful in 3 out of 8 patients and the therapy with this agent did not show any serious side effects. In summary, cyclosporine A does have potential in neuroprotective strategy and needs to be evaluated experimentally and in clinical trials.

Tacrolimus/FK506: FK506 is an immunosuppressant introduced in 1990s and the mechanism of action is similar to that of cyclosporine A. FK506 binds with FK506 binding protein (FKBP), an immunophilin, and makes a complex which results in inhibition of downstream activation of nuclear factor of

activated T-cells (NFAT), hence controlling relevant immunological genes[91].

In *in vitro* studies, FK506 has shown protection against excitotoxic neuronal death in cortical cell cultures[92]. In another *in vitro* study, FK506 protected the cultured cortical neurons against oxygen glucose deprivation. The action was inhibited in the presence of an antibody against FKBP-12 suggesting that FK506 acts via FKBP-12[93].

FK506 has been shown to provide protection in rodent model of focal ischaemia and this neuroprotection was thought to be mediated by immunophillins[11], but in subsequent study done in gerbils in transient global ischaemia model, FK506 reduced delayed neuronal death and this effect was not mediated by inhibition of neuronal NO synthase, again binding to physiological target was anticipated[94].

In primate model of cerebral ischaemia, FK506 showed a significant improvement in neurological deficits[95]. FK506 has been shown to have neuroprotective effects on cultured neurons and also in experimental models of ischaemic stroke, still a mechanistic pathway for its neuroprotective effect of tacrolimus needs to be explored.

Rapamycin: Rapamycin is a macrolide antibiotic developed as an antifungal agent but was later discovered to have potent immunosuppressive and anti-proliferative properties. Rapamycin is an immunophillin ligand that structurally resembles FK506. Unlike FK506 and cyclosporine A, rapamycin binds to mammalian targets of rapamycin (mTOR) and prevents phosphorylation of p70S6K, 4EBP1 and other proteins involved in transcription, translation and cell cycle control[96]. Rapamycin also antagonizes the anti-apoptotic signals mediated by mTOR[97], and induces autophagy and maintains normal cell metabolism[98].

Literature is available stating the benefitting effects of rapamycin in *in vitro* and *in vivo* models of cerebral ischaemia. An *in vitro* study reported that application of rapamycin at different concentrations did not affect the bioelectrical activity and evoked field excitatory post-synaptic potentials magnitude in hippocampal slices[99] suggesting an alternative to calcineurin inhibitors in events of neurotoxicity. Rapamycin treatment inhibits the hypoxia induced expression of inducible NO synthase in microglial cells[100]. In mice after experimental traumatic brain injury, rapamycin significantly improved the functional recovery[101]. Anti-depressant like effects of rapamycin in rodents have also been reported suggesting a role in treatment of affective disorders[102]. Effects of rapamycin in experimental models of cerebral ischaemia have not been systematically investigated. More studies have to be undertaken to fully acknowledge the neuroprotective significance of rapamycin.

Does mycophenolate mofetil (MMF) have any potential as neuroprotectant?

MMF is a prodrug converted into active mycophenolic acid in body and inhibits inosine mono phosphate dehydrogenase, a critical enzyme required in *de novo* purine biosynthesis. Inhibition of this enzyme leads to reduction in purine nucleotides and inhibition of cellular proliferation. MMF suppresses the monocytic production of both pro-inflammatory cytokines and microglial mitogen granulocyte macrophage colony stimulating factor[103]. It has also shown to prevent microglial activation as well as reduce the neuronal damage induced by excitotoxic injury in the *in vitro* model of stroke[104]. The microglial activation is an important pathway of proinflammatory cytokines generation, nitric oxide production and activation and release of proteases, which leads to neuronal damage[105].

Studies on the effects of MMF in animal models of CNS disorders are sparse. In mice model of amyotrophic lateral sclerosis, MMF treatment reduced microglial activation, improved stem cell survival and delayed the onset of neurological symptoms[106]. MMF has also shown protection against experimental autoimmune myasthenia gravis in rats[107] and has also been used against inflammatory myopathy and chronic inflammatory demylelinating polyradiculoneuropathy[108]. Thus MMF is an interesting candidate but its use in ischaemic brain diseases needs further exploration.

Effects of minocycline - an approach to ischaemic damages?

Minocycline is semisynthetic tetracycline derivative, which also possesses anti-inflammatory properties that are entirely distinct from its antimicrobial action. Studies have shown its usefulness in management of rheumatoid arthritis and osteoarthritis[109]. Minocycline has been recognized to have numerous pharmacological effects including ability to inhibit MMP, superoxide production and iNOS expression in human cartilage and murine macrophages[110,111]. Minocycline has been reported to penetrate blood brain barrier and provide neuroprotection in global ischaemia in gerbils[12] and focal ischaemia in rats[13]. In these studies the

neuroprotection by minocycline was associated with reduced activation of microglia and also inhibition of induction of IL-1b-converting enzyme (ICE) mRNA which was expressed mostly in microglia. In nanomolar concentrations it protected neurons in mixed spinal cord (SC) as well as pure microglia cultures against NMDA excititoxicity[112]. NMDA activated p38 mitogen-activated protein kinase (p38 MAPK), NO and IL- 1β release were effectively inhibited by minocycline in mixed spinal cord and pure microglia cultures[113]. Thus it indicates the direct effect of monocycline on microglia activation and proliferation.

Minocycline also demonstrated delayed mortality in transgenic R 6/2 model of Huntington disease by inhibiting caspase 1 and 3 expression as well as iNOS activity[114]. Minocycline treatment exhibited protection in MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) model of Parkinson's disease, this neuroprotection was mediated by reduction in expression of inducible NO synthase (iNOS) and caspase -1 expression[115]. Recently a placebo controlled open label clinical trial of minocycline in acute stroke has shown significant beneficial effects[116]. To the best of our knowledge three clinical trials are under investigation to evaluate the effects of minocycline in acute ischaemic stroke[117]. In one study, safe and well tolerated doses of minocycline are being evaluated whereas in other study minocycline, enoxaparin or combination of both will be studied on acute ischaemic stroke patients. The effect of minocycline on neurological deficits and functional outcomes in acute ischaemic stroke patients will be assessed in the other enlisted clinical trial[117].

Conclusion

Cerebral ischaemia is a multifactorial disorder which includes a number of pathways for progression of injury to brain cells. Activation of glutamate receptors, release of NO, proteases and generation of free radicals are important mechanisms in ischaemia. Inflammation is considered a major component in disseminating the detrimental effects of cerebral ischaemia. Circumscription of inflammatory cascade is an integral part in ameliorating the harmful consequences in cerebral ischaemia. In this respect, immunosuppressive agents can prove to be valuable drugs in attenuating CNS damage following ischaemia.

Immunosuppressive agents have been shown to undermine the CNS damage following ischaemia by improved neuronal survival and inhibition of mediators of inflammation in various experimental models. *In vitro* experiments using immunosuppressive agents have demonstrated the blockade of excitotoxic insult induced by NMDA as well as inhibition of microglia cells, thus inhibiting inflammatory cascade. *In vivo*, these have been shown to improve the neurological functions as seen by neurological assessment paradigms and reductions in infarct volume.

More research is required before any definitive conclusion is derived for immunosuppressive agents as neuroprotectants. Minocycline is the only drug which is being evaluated in clinical trials. An important issue that needs to be addressed is: can there be a differential dose that causes neuroprotective effect than that required to cause immunosuppression? If these drugs exhibit neuroprotection at much lower doses than that required for preventing organ transplant rejection, it may be a potential addition to the existing therapeutic armamentarium for ischaemic stroke. This will require well designed clinical trials to evaluate short as well as long term clinical outcomes *vis-a-vis* side effect profile of each drug and translate it into improvement in quality of life of stroke patients.

References

1. Bonita R. Stroke prevention: A global perspective. In: Norris JW, Hachinski V, editors. *Stroke prevention*. New York, NY: Oxford University Press; 2001. p. 259-74.
2. Truelsen T, Begg S, Mathers C. The global burden of cerebrovascular Disease. Available from: *http://www.who.int/healthinfo/statistics/bod_cerebrovasculardiseasestroke.pdf*, accessed on January 16, 2011.
3. Adams HP, Adams R, del Zoppo G, Goldstein LB. Guidelines for the early management of patients with ischaemic stroke: 2005 guidelines update. *Stroke* 2005; *36* : 916-21.
4. Külkens S, Hacke W. Thrombolysis with alteplase for acute ischaemic stroke: review of SITS-MOST and other Phase IV studies. *Expert Rev Neurother* 2007; *7* : 783-8.
5. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. *N Engl J Med* 1995; *333* : 1581-7.
6. Tsirka SE, Rogove AD, Bugge TH, Degen JL, Strickland S. An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus. *J Neurosci* 1997; *17* : 543-52.
7. Wang YF, Tsirka SE, Strickland S, Stieg PE, Soriano SG, Lipton SA. Tissue plasminogen activator (tPA) increases neuronal damage after focal cerebral ischemia in wild-type and tPA-deficient mice. *Nat Med* 1998; *4* : 228-31.
8. Zhuo M, Holtzman DM, Li Y, Osaka H, DeMaro J, Jacquin M, *et al*. Role of tissue plasminogen activator receptor LRP in hippocampal long-term potentiation. *J Neurosci* 2000; *20* : 542-9.

9. Capano M, Virji S, Crompton M. Cyclophilin-A is involved in excitotoxin-induced caspase activation in rat neuronal B50 cells. *Biochem J* 2002; *363* : 29-36.
10. Chen J, Adachi N, Tsubota S, Nagaro T, Arai T. Dexamethasone augments ischemia-induced extracellular accumulation of glutamate in gerbil hippocampus. *Eur J Pharmacol* 1998; *347* : 67-70.
11. Sharkey J, Butcher SP. Immunophilins mediate the neuroprotective effects of FK506 in focal cerebral ischemia. *Nature* 1994; *371* : 336-9.
12. Yrjänheikki J, Keinänen R, Pellikka M, Hökfelt T, Koistinaho J. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci USA* 1998; *95* : 15769-74.
13. Yrjänheikki J, Tikka T, Keinänen R, Goldsteins G, Chan PH, Koistinaho J. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci USA* 1999; *96* : 13496-500.
14. Lipton P. Ischemic cell death in brain neurons. *Physiol Rev* 1999; *79* : 1431-568.
15. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 1999; *22* : 391-7.
16. Rao AM, Hatcher JF, Kindy MS, Dempsey RJ. Arachidonic acid and leukotriene C4: role in transient cerebral ischemia of gerbils. *Neurochem Res* 1999; *24* : 1225-32.
17. Phillis JW, O'Regan MH. The role of phospholipases, cyclooxygenases, and lipoxygenases in cerebral ischemic/ traumatic injuries. *Crit Rev Neurobiol* 2003; *15* : 61-90.
18. Mattson MP, Culmsee C, Yu ZF. Apoptotic and antiapoptotic mechanisms in stroke. *Cell Tissue Res* 2000; *301* : 173-87.
19. Bandera E, Botteri M, Minelli C, Sutton A, Abrams KR, Latronico N. Cerebral blood flow threshold of ischaemic penumbra and infarct core in acute ischaemic stroke: a systematic review. *Stroke* 2006; *37* : 1334-9.
20. Graham SH, Chen J. Programmed cell death in cerebral ischemia. *J Cereb Blood Flow Metab* 2001; *21* : 99-109.
21. Weinberger JM. Evolving therapeutic approaches to treating acute ischemic stroke. *J Neurol Sci* 2006; *249* : 101-9.
22. Chan PH. Reactive oxygen radicals in signaling and damage in the ischaemic brain. *J Cereb Blood Flow Metab* 2001; *21* : 2-14.
23. Globus MY-T, Busto R, Lin B, Schnippering H, Ginsberg MD. Detection of free radical activity during transient global ischemia and recirculation: effects of intraischemic brain temperature modulation. *J Neurochem* 1995; *65* : 1250-6.
24. Fonnum F. Glutamate: A neurotransmitter in the mammalian brain. *J Neurochem* 1984; *42* : 1-11.
25. Endres M, Dirnagl U. Ischemia and stroke. *Adv Exp Med Biol* 2002; *513* : 455-73.
26. Choi DW, Koh JY, Peters S. Pharmacology of glutamate neurotoxicity in cortical cell culture: attenuation by NMDA antagonists. *J Neurosci* 1988; *8* : 185-9.
27. White BC, Sullivan JM, DeGracia DJ, O'Neil BJ, Neumar RW, Grossman LI, *et al*. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. *J Neurol Sci* 2000; *179* : 1-33.
28. Graham SH, Hickey RW. Molecular pathophysiology of stroke. In: Neuropsychopharmacology: The Fifth Generation of Progress. Davis KL, Charney D, Coyle JT, Nemeroff C, editors. Nashville TN: American College of Neuropsychopharmacology 2002.
29. Clark GD, Happel LT, Zorumski CF, Bazann NG. Enhancement of hippocampal excitatory synaptic transmission by platelet-activating factor. *Neuron* 1992; *9* : 1211-6.
30. Ying W, Han SK, Miller JW, Swanson RA. Acidosis potentiates oxidative neuronal death by multiple mechanisms. *J Neurochem* 1999; *73* : 1549-56.
31. Nagata S. Apoptosis regulated by a death factor and its receptor: Fas ligand and Fas. *Philos Trans R Soc Lond B Biol Sci* 1994; *345* : 281-7.
32. Beutler B, Bazzoni F. TNF, apoptosis and autoimmunity: a common thread? *Blood Cells Mol Dis* 1998; *24* : 216-30.
33. Yin XM, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, *et al*. Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. *Nature* 1999; *400* : 886-91.
34. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, *et al*. Cytochrome c and DATP-dependent formation of APAF-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 1997; *91* : 479-89.
35. Zukin RS, Jover T, Yokota H, Calderone A, Simionescu M, Lau C-Y. Molecular and cellular mechanisms of ischemia-induced neuronal death. In: Mohr JP, Choi DW, Grotta JC, Weir B, Wolf PA, editors. *Stroke: pathophysiology, diagnosis, and management*. Philadelphia: The Curtis Center; 2004. p. 829-54.
36. Gupta YK, Gupta M, Kohli K. Neuroprotective role of melatonin in oxidative stress vulnerable brain. *Indian J Physiol Pharmacol* 2003; *47* : 373-86.
37. Gupta S, Gupta YK, Sharma SS. Protective effect of pifithrin-alpha on brain ischaemic reperfusion injury induced by bilateral common carotid arteries occlusion in gerbils. *Indian J Physiol Pharmacol* 2007; *51* : 62-8.
38. Kochanek PM, Hallenbeck JM. Polymorphonuclear leukocytes and monocytes/macrophages in the pathogenesis of cerebral ischemia and stroke. *Stroke* 1992; *23* : 1367-79.
39. Ridet JL, Malhotra SK, Privat A, Gage FH. Reactive astrocytes: cellular and molecular cues to biological function. *Trends Neurosci* 1997; *20* : 570-7.
40. Swanson RA, Ying W, Kauppinen TM. Astrocyte influences on ischaemic neuronal death. *Curr Mol Med* 2004; *4* : 193-205.
41. Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. *Glia* 2005; *50* : 427-34.
42. Danton GH, Dietrich WD. Inflammatory mechanisms after ischemia and stroke. *J Neuropathol Exp Neurol* 2003; *62* : 127-36.
43. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 1999; *22* : 391-7.
44. Polazzi E, Contestabile A. Reciprocal interactions between microglia and neurons: from survival to neuropathology. *Rev Neurosci* 2002; *13* : 221-42.

45. Wirjatijasa F, Dehghani F, Blaheta RA, Korf HW, Hailer NP. Interleukin-4, interleukin-10, and interleukin-1-receptor antagonist but not transforming growth factor-beta induce ramification and reduce adhesion molecule expression of rat microglial cells. *J Neurosci Res* 2002; *68* : 579-87.

46. Woodroofe MN, Sarna GS, Wadhwa M, Hayes GM, Loughlin AJ, Tinker A, *et al*. Detection of interleukin-1 and interleukin-6 in adult rat brain, following mechanical injury, by *in vivo* microdialysis: evidence of a role for microglia in cytokine production. *J Neuroimmunol*1991; *33* : 227-36.

47. Lee SC, Liu W, Dickson DW, Brosnan CF, Berman JW. Cytokine production by human fetal microglia and astrocytes. Differential induction by lipopolysaccharide and IL-1 beta. *J Immunol* 1993; *150* : 2659-67.

48. Tsirka, S.E. Clinical implications of the involvement of tPA in neuronal cell death. *J Mol Med* 1997; *75* : 341-7.

49. del Zoppo GJ, Milner R, Mabuchi T, Hung S, Wang X, Berg GI, *et al*. Microglial activation and matrix protease generation during focal cerebral ischemia. *Stroke* 2007; *38* : 646-51.

50. Boje KM, Arora PK. Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. *Brain Res* 1992; *587* : 250-6.

51. Marler JR, Goldstein LB. Medicine. Stroke-tPA and the clinic. *Science* 2003; *301* : 1677.

52. Rieckmann P. Imatinib buys time for brain after stroke. *Nat Med* 2008; *14* : 712-3.

53. Gupta YK, Briyal S, Sharma U, Jagannathan NR, Gulati A. Effect of endothelin antagonist (TAK-044) on cerebral ischaemic volume, oxidative stress markers and neurobehavioral parameters in the middle cerebral artery occlusion model of stroke in rats. *Life Sci* 2005; 77 : 15-27.

54. Briyal S, Pant AB, Gupta YK. Protective effect of endothelin antagonist (TAK-044) on neuronal cell viability in *in vitro* oxygen-glucose deprivation model of stroke. *Indian J Physiol Pharmacol* 2006; *50* : 157-62.

55. Chaudhary G, Sinha K, Gupta YK. Protective effect of exogenous administration of alpha-tocopherol in middle cerebral artery occlusion model of cerebral ischemia in rats. *Fundam Clin Pharmacol* 2003; *17* : 703-7.

56. Sinha K, Chaudhary G, Gupta YK. Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life Sci* 2002; *71* : 655-65.

57. Sinha K, Degaonkar MN, Jagannathan NR, Gupta YK. Effect of melatonin on ischemia reperfusion injury induced by middle cerebral artery occlusion in rats. *Eur J Pharmacol* 2001; *428* : 185-92.

58. Vaughan CJ, Delanty N. Neuroprotective properties of statins in cerebral ischemia and stroke. *Stroke* 1999; *30* : 1969-73.

59. Endres M, Laufs U, Huang Z, Nakamura T, Huang P, Moskowitz MA, *et al*. Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 1998; *95* : 8880-5.

60. Amin-Hanjani S, Stagliano NE, Yamada M, Huang PL, Liao JK, Moskowitz MA. Mevastatin, an HMG-CoA reductase inhibitor, reduces stroke damage and upregulates endothelial nitric oxide synthase in mice. *Stroke* 2001; *32* : 980-6.

61. Newcomb JD, Ajmo CT, Jr., Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE. Timing of cord blood treatment after experimental stroke determines therapeutic efficacy. *Cell Transplant* 2006; *15* : 213-23.

62. Vendrame M, Cassady J, Newcomb J, Butler T, Pennypacker KR, Zigova T, *et al*. Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. *Stroke* 2004; *35* : 2390-5.

63. Su EJ, Fredriksson L, Geyer M, Folestad E, Cale J, Andrae J, *et al*. Activation of PDGF-CC by tissue plasminogen activator impairs blood-brain barrier integrity during ischaemic stroke. *Nat Med* 2008; *14* : 731-7.

64. Hailer NP. Immunosuppression after traumatic or ischaemic CNS damage: it is neuroprotective and illuminates the role of microglial cells. *Prog Neurobiol* 2008; *84* : 211-33.

65. Betz AL, Coester HC. Effect of steroids on edema and sodium uptake of the brain during focal ischemia in rats. *Stroke* 1990; *21* : 1199-204.

66. Zhou Y, Ling EA, Dheen ST. Dexamethasone suppresses monocyte chemoattractant protein-1 production via mitogen activated protein kinase phosphatase-1 dependent inhibition of Jun N-terminal kinase and p38 mitogen-activated protein kinase in activated rat microglia. *J Neurochem* 2007; *102* : 667-78.

67. Schumacher M. Rapid membrane effects of steroid hormones: an emerging concept in neuroendocrinology. *Trends Neurosci* 1990; *13* : 359-62.

68. Tanaka J, Fujita H, Matsuda S, Toku K, Sakanaka M, Maeda N. Glucocorticoid- and mineralocorticoid receptors in microglial cells: the two receptors mediate differential effects of corticosteroids. *Glia* 1997; *20* : 23-37.

69. Chao CC, Hu S, Close K, Choi CS, Molitor TW, Novick WJ, *et al*. Cytokine release from microglia: differential inhibition by pentoxifylline and dexamethasone. *J Infect Dis* 1992; *166* : 847-53.

70. Drew PD, Chavis JA. Inhibition of microglial cell activation by cortisol. *Brain Res Bull* 2000; *52* : 391-6.

71. Jacobsson J, Persson M, Hansson E, Ronnback L. Corticosterone inhibits expression of the microglial glutamate transporter GLT-1 *in vitro*. *Neuroscience* 2006; *139* : 475-83.

72. Bertorelli R, Adami M, Di Santo E, Ghezzi P. MK 801 and dexamethasone reduce both tumor necrosis factor levels and infarct volume after focal cerebral ischemia in the rat brain. *Neurosci Lett* 1998; *246* : 41-4.

73. Fenske A, Fischer M, Regli F, Hase U. The response of focal ischaemic cerebral edema to dexamethasone. *J Neurol* 1979; *220* : 199-209.

74. de Courten-Myers GM, Kleinholz M, Wagner KR, Xi G, Myers RE. Efficacious experimental stroke treatment with high-dose methylprednisolone. *Stroke* 1994; *25* : 487-92.

75. De Reuck J, Vandekerckhove T, Bosma G, De Meulemeester K, Van Landegem W, De Waele J, *et al*. Steroid treatment in acute ischaemic stroke. A comparative retrospective study of 556 cases. *Eur Neurol* 1988; *28* : 70-2.

76. Norris JW. Steroid therapy in acute cerebral infarction. *Arch Neurol* 1976; *33* : 69-71.

77. Butcher SP, Henshall DC, Teramura Y, Iwasaki K, Sharkey J. Neuroprotective actions of FK506 in experimental stroke: *in vivo* evidence against an antiexcitotoxic mechanism. *J Neurosci* 1997; *17* : 6939-46.

78. Mulkey RM, Endo S, Shenolikar S, Malenka RC. Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. *Nature* 1994; *369* : 486-8.

79. Nichols RA, Suplick GR, Brown JM. Calcineurin-mediated protein dephosphorylation in brain nerve terminals regulates the release of glutamate. *J Biol Chem* 1994; *269* : 23817-23.

80. Xin G, Dillman JF, Valina LD, Dawson TM. Neuroimmunophilins: novel neuroprotective and neuroregenerative targets. *Ann Neurol* 2001; *50* : 6-16.

81. Elliott JF, Lin Y, Mizel SB, Bleackley RC, Harnish DG, Paetkau V. Induction of interleukin 2 messenger RNA inhibited by cyclosporin A. *Science* 1984; *226* : 1439-41.

82. Reem GH, Cook LA, Vilcek J. Gamma interferon synthesis by human thymocytes and T lymphocytes inhibited by cyclosporin A. *Science* 1983; 221 : 63-5

83. Soriano-Izquierdo A, Gironella M, Massaguer A, Salas A, Gil F, Piqué JM, *et al*. Effect of cyclosporin A on cell adhesion molecules and leukocyte-endothelial cell interactions in experimental colitis. *Inflamm Bowel Dis* 2004; *10* : 789-800.

84. Sheehan J, Eischeid A, Saunders R, Pouratian N. Potentiation of neurite outgrowth and reduction of apoptosis by immunosuppressive agents: implications for neuronal injury and transplantation. *Neurosurg Focus* 2006; *20* : E9.

85. Shiga Y, Onodera H, Matsuo Y, Kogure K. Cyclosporin A protects against ischemia-reperfusion injury in the brain. *Brain Res* 1992; *595*; 145-8.

86. Uchino H, Elmer E, Uchino K, Lindvall O, Siesjo BK. Cyclosporine A dramatically ameliorates CA1 hippocampal damage following transient forebrain ischemia in the rat. *Acta Physiol Scand* 1995; *155* : 469-71.

87. Miyata K, Omori N, Uchino H, Yamaguchi T, Isshiki A, Shibasaki F. Involvement of the brain-derived neurotrophic factor/TrkB pathway in neuroprotecive effect of cyclosporin A in forebrain ischemia. *Neuroscience* 2001; *105* : 571-8

88. Wakita H, Tomimoto H, Akiguchi I, Kimura J. Protective effect of cyclosporin A on white matter changes in the rat brain after chronic cerebral hypoperfusion. *Stroke* 1995; *26* : 1415-22.

89. Drachman DB, Adams RN, McIntosh K, Pestronk A. Treatment of experimental myasthenia gravis with cyclosporin A. *Clin Immunol Immunopathol* 1985; *34* : 174-88.

90. Mahattanakul W, Crawford TO, Griffin JW, Goldstein JM, Cornblath DR. Treatment of chronic inflammatory demyelinating polyneuropathy with cyclosporin-A. *J Neurol Neurosurg Psychiatry* 1996; *60* : 185-7.

91. Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* 1997; *15* : 707-47.

92. Dawson TM, Steiner JP, Dawson VL, Dinerman JL, Uhl GR, Snyder SH. Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. *Proc Natl Acad Sci USA* 1993; *90* : 9808-12.

93. Labrande C, Velly L, Canolle B, Guillet B, Masmejean F, Nieoullon A, *et al*. Neuroprotective effects of tacrolimus (FK506) in a model of ischaemic cortical cell cultures: role of glutamate uptake and FK506 binding protein 12 kDa. *Neuroscience* 2006; *137* : 231-9.

94. Tokime T, Nozaki K, Kikuchi H. Neuroprotective effect of FK506, an immunosuppressant, on transient global ischemia in gerbil. *Neurosci Lett* 1996; *206* : 81-4.

95. Furuichi Y, Maeda M, Matsuoka N, Mutoh S, Yanagihara T. Therapeutic time window of tacrolimus (FK506) in a nonhuman primate stroke model: comparison with tissue plasminogen activator. *Exp Neurol* 2006; *204* : 138-46.

96. Vignot S, Faivre S, Aguirre D, Raymond E. mTOR-targeted therapy of cancer with rapamycin derivatives. *Ann Oncol* 2005; *16* : 525-37.

97. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, *et al*. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 2002; *8* : 128-35.

98. Kamada Y, Sekito T, Ohsumi Y. Autophagy in yeast: a TOR-mediated response to nutrient starvation. *Curr Top Microbiol Immunol* 2004; *279* : 73-84.

99. Daoud D, Scheld HH, Speckmann EJ, Gorji A. Rapamycin: brain excitability studied *in vitro*. *Epilepsia* 2007; *48* : 834-6.

100. Lu DY, Liou HC, Tang CH, Fu WM. Hypoxia-induced iNOS expression in microglia is regulated by the PI3-kinase/Akt/mTOR signaling pathway and activation of hypoxia inducible factor-1alpha. *Biochem Pharmacol* 2006; *72* : 992-1000.

101. Erlich S, Alexandrovich A, Shohami E, Pinkas-Kramarski, R. Rapamycin is a neuroprotective treatment for traumatic brain injury. *Neurobiol Dis* 2007; *26* : 86-93.

102. Cleary C, Linde JA, Hiscock KM, Hadas I, Belmaker RH, Agam G, *et al*. Antidepressive-like effects of rapamycin in animal models: Implications for mTOR inhibition as a new target for treatment of affective disorders. *Brain Res Bull* 2008; *76* : 469-73

103. Nagy SE, Andersson JP, Andersson UG. Effect of mycophenolate mofetil (RS-61443) on cytokine production: inhibition of super antigen induced cytokines. *Immunopharmacology* 1993; *26* : 11-20.

104. Dehghani F, Hischebeth GT, Wirjatijasa F, Kohl A, Korf HW, Hailer NP. The immunosuppressant mycophenolate mofetil attenuates neuronal damage after excitotoxic injury in hippocampal slice cultures. *Eur J Neurosci* 2003; *18* : 1061-72.

105. Miljkovic D, Samardzic T, Cvetkovic I, Mostarica SM, Trajkovic V. Mycophenolic acid down regulates inducible nitric oxide synthase induction in astrocytes. *Glia* 2002; *39* : 247-55.

106. Yan J, Xu L,Welsh AM, Chen D, Hazel T, Johe K, *et al*. Combined immunosuppressive agents or CD4 antibodies prolong survival of human neural stem cell grafts and improve disease outcomes in amyotrophic lateral sclerosis transgenic mice. *Stem Cells* 2006; *24* :1976-85.

107. Janssen SP, Phernambucq M, Martinez-Martinez P, De Baets MH, Losen M. Immunosuppression of experimental autoimmune myasthenia gravis by mycophenolate mofetil. *J Neuroimmunol* 2008; *201-202* : 111-20.

108. Chaudhry V, Cornblath DR, Griffin JW, O'Brien R, Drachman DB. Mycophenolate mofetil: a safe and promising immunosuppressant in neuromuscular diseases. *Neurology* 2001; *56* : 94-6

109. Ryan ME, Greenwald RA, Golub LM. Potential of tetracyclines to modify cartilage breakdown in osteoarthritis. *Curr Opin Rheumatol* 1996; *8* : 238-47.

110. Golub LM, Lee HM, Ryan ME, Giannobile WV, Payne J, Sorsa T. Tetracyclines inhibit connective tissues breakdown by multiple non-antimicrobial mechanisms. *Adv Dental Res* 1998; *12* : 12-26.

111. Gabler WL, Creamer HR. Suppression of human neutrophil functions by tetracyclines. *J Periodontal Res* 1991; *26* : 52-8.

112. Tikka TM, Koistinaho JE. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol* 2001; *166* : 7527-33.

113. Tikka T, Fiebich BL, Goldsteins G, Keinanen R, Koistinaho J. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J Neurosci* 2001; *21* : 2580-8.

114. Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, *et al*. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med* 2000; *6* : 797-801.

115. Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, *et al*. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *PNAS* 2001; *98* : 14669-74.

116. Lampl Y, Boaz M, Gilad R, Lorberboym M, Dabby R, Rapoport A, *et al*. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. *Neurology* 2007; *69* : 1404-10.

117. www.clinicaltrials.gov, accessed on January 16, 2011.

Reprint requests: Dr Y.K. Gupta, Professor & Head, Department of Pharmacology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India
e-mail: yk.ykgupta@gmail.com