Neuroprotective FK506 Does Not Alter In Vivo Nitric Oxide Production During Ischemia and Early Reperfusion in Rats

Thomas J. Toug, MD; Anish Bhardwaj, MD; Valina L. Dawson, PhD; Ted M. Dawson, MD, PhD; Richard J. Traystman, PhD; Patricia D. Hurn, PhD

Background and Purpose—Previous studies have demonstrated that the immunosuppressant FK506 provides neuroprotection in experimental brain injury and suggest that this action may be mediated by suppression of neuronal nitric oxide synthase activation that occurs after ischemic depolarization. We sought to determine whether FK506 reduces histological injury after middle cerebral artery occlusion (MCAO) in the rat and whether the neuroprotective effect is mediated via suppression of in vivo nitric oxide (NO) production during ischemia or early reperfusion.

Methods—Under controlled conditions of normoxia, normocarbia, and normothermia, halothane-anesthetized male Wistar rats were subjected to 2 hours of MCAO by the intraluminal occlusion technique in a blinded, randomized experimental trial. Ipsilateral parietal cortical laser-Doppler flowmetry was monitored throughout ischemia. Animals were randomly assigned to 4 pretreatment groups: intravenous FK506 0.3 mg/kg or 1.0 mg/kg, vehicle (cremaphor), or an equivalent volume of saline administered 30 minutes before MCAO. Infarction volume was assessed by a triphenyltetrazolium chloride staining at 22 hours of reperfusion. In separate experiments, microdialysis probes were placed bilaterally into the striatum. Rats were perfused with artificial cerebrospinal fluid containing 3 μmol/L [14C]-L-arginine for 3 hours and then subjected to 2 hours of right MCAO. Intravenous 0.3 mg/kg FK506 or cremaphor was given 30 minutes before right MCAO. Right-left differences between [14C]-L-citrulline in the effluent were assumed to reflect differences in NO production.

Results—All values are mean ± SE. FK506 at 0.3 mg/kg reduced infarction volume in cortex: 40 ± 12 mm³ compared with saline (109 ± 15 mm³) and cremaphor (148 ± 23) (P < 0.05). Striatal infarction was also reduced by low-dose FK506: 16 ± 4 mm³ versus 36 ± 4 mm³ and 34 ± 4 mm³ in saline and vehicle groups, respectively (P < 0.05). High-dose treatment reduced infarction volume in cortex (61 ± 14 mm³, P < 0.05 from saline and vehicle groups) and in striatum (22 ± 5 mm³, P < 0.05 from saline and vehicle groups). [14C]-L-citrulline recovery via microdialysis was markedly enhanced in ischemic compared with nonischemic striatum. However, ischemia-evoked [14C]-L-citrulline recovery was not different in FK506-treated rats compared with vehicle-treated animals.

Conclusions—These data demonstrate that FK506 provides robust neuroprotection against transient focal cerebral ischemia in the rat. The mechanism of protection in vivo is not through attenuation of ischemia-evoked NO production during MCAO and early reperfusion. (Stroke. 1999;30:1279-1285.)

Key Words: immunosuppressive agents ■ microdialysis ■ nitric oxide ■ stroke ■ rats

FK506 (tacrolimus) is a macrocyclic lactone with potent immunosuppressive properties. Previous studies have demonstrated a significant neuroprotective action of FK506 in experimental models of cerebral ischemia, suggesting a novel therapeutic application of this drug.1–6 The cellular mechanisms underlying this protective action remain unclear. The mechanism of the immunosuppressive action of FK506 involves the inhibition of a calcium-dependent phosphatase, calcineurin, by a complex of FK506 and the 12-kDa immunophilin FBKP12 (FK506 binding protein).1,2 FBKP12 is distributed abundantly in the central nervous system and colocalizes with neuronal calcineurin.8,9 In vitro studies have demonstrated that FK506 protects primary cortical cell cultures against N-methyl-D-aspartate (NMDA)-induced neurotoxicity, suggesting a potential direct neuroprotective action.10 NMDA toxicity is well known to be mediated in part by nitric oxide (NO),10–12 in that cell injury is blocked by various inhibitors of nitric oxide synthase (NOS). Further,
NOS activity is regulated by its phosphorylation state in that dephosphorylation increases catalytic activity. FK506 enhances NOS phosphorylation, inhibiting subsequent catalytic activity as quantified by decreases in cortical cGMP levels and NO breakdown products. One hypothesis is that FK506 acts in vitro as a neuroprotectant, presumably through calcineurin inhibition, by preventing dephosphorylation/activation of NOS and subsequent NO mediated toxicity. The purpose of the present study was to determine whether FK506 improves histological injury after reversible middle cerebral artery occlusion (MCAO), whether the observed protection is dose dependent, and whether the mechanism is linked to attenuation of acute, ischemia-evoked NO production in vivo.

Materials and Methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of The Johns Hopkins University and conforms to the National Institutes of Health guidelines for the care and use of animals in research. All methods are as previously reported. In brief, male Wistar rats (250 to 420 g; Harlan, Indianapolis, Ind) were anesthetized with 1% to 2% halothane delivered via face mask in oxygen-enriched air and instrumented with femoral artery catheters for physiological monitoring and blood gas measurement. Rectal temperatures were controlled at approximately 37°C using heat lamps. Laser-Doppler flowmetry (LDF, Moor Instruments Ltd, model MBF3D) was measured to confirm vascular occlusion and reperfusion, with probe placement at 2 mm posterior and 6 mm lateral to bregma, as previously described.

Focal cerebral ischemia was accomplished using the intraluminal filament model (4-0 nylon monofilament suture) of proximal MCAO. The right common carotid artery was exposed through a lateral incision, separated from the vagus, and ligated. The external carotid artery was ligated, the occipital branch cauterized, and the pterygopalatine artery ligated. An occluding filament was advanced via common carotid artery until the LDF signal displayed an abrupt and significant reduction, confirming ongoing ischemia, then secured in place. Ischemic LDF was determined over 5-minute periods throughout the 2-hour occlusion period, then the suture was withdrawn with prompt restoration of the LDF signal. If intraischemic LDF was not sharply reduced with stabilization at less than 40% of baseline signal, MCAO was considered incomplete, and the animal was excluded from the study. Each animal was recovered and supported with intravenous saline (0.6 mL/h) and supplemental oxygen as needed. After 22 hours of reperfusion, the brain was harvested under halothane anesthesia, and the tissue was sliced into seven 2-mm-thick coronal sections for 3,3′,5-triphenyltetrazolium chloride (TTC) staining and quantification via standard photography and digital planimetry (SigmaScan Pro, Jandel). The infarcted area was numerically integrated across each section and over the entire ipsilateral hemisphere. Infarct volume was measured separately in the cortex and caudate putamen, corrected for edema as described by Lin et al. and expressed as a volume percentage of the ipsilateral structure.

NOS Activity via Microdialysis

Dialysis probes used in these studies were made as described previously. Briefly, each was a single hollow dialysis fiber sealed with epoxy at one end; membrane diameter was 300 μm, with molecular mass cutoff of 5 kDa. Two hollow silica perfusion tubes were inserted into the dialysis fiber so that their ends were 3 mm apart. The distance between the tips constitutes the effective dialyzing area of the cannula. Recovery across the dialysis probe is approximately 15% to 20% in vitro.

In experiments with MCAO accompanied by microdialysis, the animal was initially anesthetized with pentobarbital (60 mg/kg IP), then placed in a Kopf stereotaxic frame for cannula placement in striatum (0.5 mm anterior and 2.5 mm lateral to the bregma; depth, 6 mm from the dura). A 2×2-mm area of the skull was removed with a variable-speed drill. A thin layer of bone was left intact and removed with forceps under microscopic observation to minimize trauma to the cortex. Cannulas were advanced to predetermined coordinates with a micromanipulator and fixed in position with dental cement. The animal was then removed from the stereotaxic apparatus and allowed a postsurgical equilibration period during which pentobarbital was discontinued and anesthesia maintained for the remainder of the experiment with 1% to 2% halothane. Starting 1 hour after insertion, the cannulas were perfused at 1 μL/min with artificial cerebrospinal fluid (aCSF): NaCl 131.8, NaHCO3 24.0, CaCl2 2.0, KCl 1.26, MgCl2 0.65, urea 6.7, and dextrose 3.7 mmol/L. The aCSF was filtered, warmed to 37°C, and bubbled with 95% N2/5% CO2 until O2 and CO2 tensions were similar to aCSF and brain tissue. Microdialysis effluents were collected over 20-minute periods throughout the ischemic period and compared on a paired basis (right versus left striatum within each animal).

Striatal NO production was measured as previously described, based on modifications of an isotopic conversion assay which quantifies conversion of arginine to equimolar citrulline and NO via NOS. Therefore, right-left differences between [14C]-L-citrulline in the effluent presumably reflect differences in local NO production. During continuous infusion of aCSF containing 3 μmol/L [14C]-L-arginine, 20 μL effluent dialysate samples were collected during 20-minute periods and assayed for [14C]-L-citrulline content. Samples were diluted with 200 μL water and poured over 0.5 mL resin columns (AG-50W8X, Na+ form, pH 7.0). Columns were washed with 2 mL buffer containing 30 mmol/L HEPES (pH 5.2), 3 mmol/L EDTA, and 1 mL water. Radioactivity of flow through the column was quantified by liquid scintillation spectroscopy. To determine renin efficiency of arginine trapping, 20 μL aCSF containing 3 μmol/L [14C]-L-arginine (not used for dialysis) was diluted in 200 μL water, poured over a column, and washed as above. Specific activity was corrected for counting efficiency and background activity and expressed as femtomoles per minute of perfusion. As an internal control, 100 μL aCSF not used for dialysis was directly assayed for activity to ensure that consistent concentrations of [14C]-l-arginine were added to the aCSF.

Experimental Protocols

Validation of In Vivo NOS Activity Assay During MCAO

In initial experiments, we evaluated the capability of the microdialysis loading/sampling method to track changes in NO production over time during MCAO. Three groups of rats received initial [14C]-l-arginine pre-loading via dialysis probe for 3 hours, followed by either MCAO or sham conditions and measurements of effluents over an additional 3 hours. The [14C]-l-arginine perfusion was continued throughout the experimental period to assure adequate cell substrate availability. In 1 group (MCAO, n = 8), occlusion was induced and effluents were collected at 20-minute intervals. In a second group (sham, n = 7), animals were treated with neck surgery without introduction of the intraluminal suture. A final group received 1 mmol/L L-nitroarginine (L-NNA, n = 7) via microdialysis probe into the right striatum 1 hour before right MCAO. The inhibitor concentration was selected on the basis of our previous findings that 1 mmol/L L-NNA reduces NMDA- and AMPA-stimulated [3H]-citrulline production. The right-left effluent concentrations of [14C]-L-citrulline were compared on a paired basis in each animal. Appropriate probe placement relative to the area of TTC-determined infarction was confirmed in all cases.

FK506 and MCAO

To demonstrate the efficacy of FK506 in reducing histological injury after MCAO with reperfusion, rats were assigned in blinded fashion to 1 of 4 intravenous treatment groups: (1) 0.3 mg/kg FK506 (n = 11), (2) 1.0 mg/kg FK506 (n = 10), (3) cremophor (drug vehicle, equivalent volume) (n = 10), and (4) saline (n = 11). Dosages were chosen based on efficacy of FK506 in other ischemic models. Each infusion was given 30 minutes before MCAO over a 15-minute duration. Preliminary experiments demonstrated that FK506 at 1 mg/kg or vehicle (n = 7 per group) did not alter mean arterial pressure.
or LDF immediately on infusion or for 120 minutes after infusion (data not shown). In a separate cohort of animals, the effect of FK506 on ischemia-evoked NO production and on persistent NO production in reperfusion was evaluated. Rats were randomly assigned to receive either intravenous infusion of 0.3 mg/kg FK506 (n = 5) or cremophor vehicle (n = 4) 30 minutes before right MCAO. As before, LDF was evaluated throughout MCAO to determine whether there were differences in the reduction of LDF signal during MCAO among treatment groups. Microdialysis effluents were collected over 20-minute intervals throughout MCAO and 3 hours of reperfusion. All surgical instrumentation was removed, and the animals recovered. Rats were killed at 22 hours of reperfusion for confirmation of probe placement within the area of TTC-determined infarction.

Materials
[14C]-L-Arginine (317 mCi/mmol) was obtained from Amersham, L-NNA from Sigma Chemicals, and cremophor from BASF. FK506 was a generous gift from Fujisawa Pharmaceuticals.

Statistical Analysis
Within each group, the citrulline composition of the effluent was analyzed by 2-way ANOVA: effluent citrulline from the 2 striata (ischemic versus nonischemic) were analyzed as 1 within-subjects factor, and the 20-minute collections as a second within-subjects factor. If the overall effect of treatment or treatment × time interaction was significant, comparisons of mean values between the 2 striata at individual time points were made by orthogonal contrasts. Infarction volume and physiological data were analyzed by 1-way ANOVA. A value of P < 0.05 was considered significant. Data are presented as mean ± SEM.

Results
Validation of In Vivo NOS Activity Assay During MCAO
Physiological variables were held within normal values, and there were no differences among the 3 treatment groups before and during MCAO. Perfusion with [14C]-L-arginine in aCSF resulted in time-dependent increases in [14C]-L-citrulline in the effluent bilaterally (Figure 1), presumably reflecting transport time of labeled arginine into the tissue and transport of labeled citrulline back to the probe. During MCAO, labeled citrulline recovery rapidly increased in the ischemic striatum and continued to increase throughout our window of observation compared with the nonischemic striatum (Figure 1, top). This marked increase was lacking in sham-operated animals, and citrulline recovery was equivalent in both right and left striatum over time (Figure 1, bottom). Figure 2 demonstrates that ischemia-induced increases in labeled citrulline recovery were not observed when the ischemic striatum was pretreated with L-NNA. During right MCAO, labeled citrulline recovery in right striatum remained significantly lower for the entire 3-hour experimental period compared with the non–L-NNA-treated left striatum (Figure 2). Therefore, as expected, striatal labeled citrulline recovery (and presumably stoichiometric amounts of NO) increased over time during vascular occlusion compared with the nonischemic region. Further, this fraction was clearly inhibitable by local L-NNA treatment.

Effect of FK506 on Infarction Volume
Physiological variables were held within normal values, and there were no differences among treatment groups before MCAO, during MCAO, or during early reperfusion. The ipsilateral LDF signal during MCAO decreased rapidly to approxi-
was excluded from the study. Three animals were excluded in this manner in both the cremaphor and saline treatment groups; 2 animals were excluded in each of the FK-506 treatment groups. Averaged LDF over the ischemic period was not different among groups: saline, 20 \pm 1\% of baseline; cremaphor, 27 \pm 1\%; FK506 0.3 mg/kg, 24 \pm 1\%; and FK506 1 mg/kg, 20 \pm 1\%. Cortical infarction volume was reduced by FK506 at both the 0.3- and 1-mg/kg doses (40 \pm 12 mm\(^3\) and 61 \pm 14 mm\(^3\), respectively) compared with cremaphor vehicle (148 \pm 23 mm\(^3\), \(P\leq 0.05\)) or saline treatment (109 \pm 15 mm\(^3\), \(P\leq 0.05\)) (Figure 4).

However, infarction size was not different between drug-treated groups, indicating that both doses provided equivalent neuroprotection in cortex. In the striatum, infarction volume was again reduced by both low- and high-dose FK506 treatment (16 \pm 4 mm\(^3\) and 22 \pm 5 mm\(^3\), respectively) compared with cremaphor vehicle (34 \pm 4 mm\(^3\), \(P\leq 0.05\)) or saline treatment (36 \pm 4 mm\(^3\), \(P\leq 0.05\)) (Figure 4).

Mortality as assessed by survival to 22 hours’ reperfusion was as follows for the treatment groups: cremaphor (4/17 animals, or 24\%), saline (4/18, 22\%), FK506 0.3 mg/kg (5/18, 28\%) and FK506 1 mg/kg (5/21, 24\%).

**Effect of FK506 on NO Production During MCAO and Reperfusion**

Arterial blood pressure, blood gases, and rectal temperature were within normal physiological range and not different in FK506- or vehicle-treated animals. As in the previous protocol, reduction of ischemic LDF was not different in drug- and vehicle-treated groups. On withdrawal of the monofilament, LDF restored...
rapidly to baseline values within 15 minutes in both groups. As expected, there was a time-dependent increase in labeled citrulline recovery in the striatum bilaterally in all animals (Figure 5). FK506 treatment did not significantly affect labeled citrulline recovery in either ischemic or nonischemic striatum. Compared with nonischemic striatum, labeled citrulline recovery increased over the 2 hours of MCAO and continued to increase throughout reperfusion in both FK506 (Figure 5, top) or cremaphor vehicle–treated (bottom) animals.

**Discussion**

This study demonstrates 2 important findings. First, pretreatment with FK506 provides robust neuroprotection during reversible MCAO in the rat. Both doses tested in this model provide equivalent reduction of injury at 22 hours of reperfusion. Second, the compound’s neuroprotective mechanism is not linked to suppression of NOS activity during ischemia or early reperfusion, as measured in vivo by labeled citrulline recovery, and reduction of NO toxicity.

FK506 has been previously shown to have significant neuroprotective properties. In vitro work in primary cortical culture demonstrated that the agent strongly reduces NMDA, but not non-NMDA, neurotoxicity. FK506 also proved to be protective in several models of global cerebral ischemia. Further, FK506 0.1 mg/kg reduced tissue infarction after localized, perivascular endothelin administration and consequent loss of regional perfusion, even when administered 60 minutes after the insult. Using a standard experimental model of reversible MCAO, we also observed that FK506 pretreatment reduces injury over a range of doses (0.3 to 1 mg/kg).

Because FK506 has numerous mechanisms of action within neurons and inflammatory cells, its protective action is likely multifactorial. FK506 is known to modify immunologic reactions by suppression of interleukin-2 gene expression and inhibit protein phosphorylation. FK506, complexed with FKBP, inhibits the function of calcineurin thereby inhibiting Ca²⁺–dependent dephosphorylation and activation of neuronal NOS. We hypothesized that FK506 treatment could suppress the activation of neuronal NOS, which ordinarily occurs subsequent to ischemic depolarization, NMDA receptor activation, and resulting rise in intracellular cation flux. If FK506 enhanced NOS phosphorylation in vivo, thus inhibiting the enzyme’s catalytic activity during ischemia and early reperfusion, decreased arginine metabolism would be quantifiable as lower labeled citrulline recovery from drug–versus vehicle-treated tissue. However, the rise in striatal citrulline recovery (in femtomoles per minute) during MCAO in untreated animals was also observed with FK506 pretreatment at a dose that reduced striatal infarction. Therefore, the drug had little effect on ischemic NOS catalytic activity, at least within this brain region. It must be emphasized that our dynamic but indirect measurement of NOS activity (arginine-to-citrulline conversion) measures total NOS activity in the dialysate without distinction among contributions from various enzyme isoforms. Because we measured NOS activity during severe focal ischemia up to 6 hours after MCAO, we think it likely that neuronal and endothelial sources contribute most greatly to our signal. The finding that early, ischemia-induced NOS activation is not altered by FK506 treatment is consistent with the observation that the compound protects against NMDA toxicity in cortical neurons cultured from nNOS-deficient mice to the same extent as in wild-type cultures.

FK506 has also been reported to suppress inducible NOS (iNOS) production in cultured macrophages, and ischemia-induced iNOS expression is elevated in reactive astrocytes in some brain regions for days after even brief ischemia. Although we detect uniform increases in labeled citrulline recovery within 1 hour of MCAO, relatively little iNOS is elaborated from inflammatory cells by very early reperfusion. Therefore, our measurements of ischemia-evoked NOS production do not address iNOS-mediated mechanisms that may be activated beyond the first 3 hours of reperfusion. Therefore, iNOS could be involved in FK506-induced neuroprotection in the maturing lesion.

Several alternative neuroprotective mechanisms must be considered for the ability of FK506 to salvage tissue during vascular occlusion. Nonimmunosuppressive properties of FK506 may be relevant. FK506 analogs without immunosuppressive activity promote neurite growth and enhance morphological and functional recovery after peripheral nerve injury, as does FK506. Glutamate toxicity and Ca²⁺ influx–initiated intracellular events have been well studied and likely lead to protein kinase redistribution within cell organelles and enhanced tyrosine phosphorylation of synaptic proteins. Because FK506 inhibits the phosphatase calcineurin, the drug could restore fundamental imbalances in neuronal protein phosphorylation during reperfusion or play a role in Ca²⁺–triggered apoptosis. Intracellular calcium homeostasis may be altered through FK506-sensitive ryanodine and ionositol 1,4,5-triphosphate receptor–associated calcium ion channels within endoplasmic reticulum and cell membranes. Finally, oxidative stress is known to result in impaired mitochondrial function; the pathophysiology of the injury is thought to involve the formation of inner mitochondrial membrane pores through which protons and possibly calcium ions are released. Cyclosporin A, a compound related to FK506, has been shown to prevent or impede pore formation under conditions of oxidative stress accompanied by high-calcium load. Thus, the importance of FK506 in protection from free radical–induced mitochondrial transition states should also be considered. FK506 at doses similar to those of the present study prevents secondary deterioration of mitochondrial function in penumbral areas after MCAO and improves postischemic respiratory rates in vitro.

As confirmed by postmortem dissection, all microdialysis probe tips were localized in caudate nucleus. The choice of striatum as a measurement site is based on its known vulnerability to ischemia and excitotoxic glutamatergic injury. Using well-characterized antibodies, we have confirmed an abundance of neuronal and endothelial NOS isoforms in striatum and assume abundant NO synthesis in the area. Utilizing microdialysis capture of labeled citrulline, we previously demonstrated that NMDA and AMPA stimulate NO production in rat hippocampus and lamb neocortex. We have now shown that MCAO increases citrulline recovery in striatum and confirmed that recovery is attenuated by the NOS inhibitor L-NNA. Therefore, ischemia-evoked increases in radiolabeled citrulline recovery are assumed to reflect increased NO production. However, it must be emphasized that citrulline recovery is...
actually an indirect marker of NO production in vivo because of complex compartmental kinetics. For example, the time-dependent increase in labeled citrulline recovery under control conditions likely reflect these kinetics rather than increased NO synthesis over time.\textsuperscript{17,19} Bilateral microdialysis perfusion was used in a paired experimental design to reduce interanimal variability.

Some differences in absolute levels of basal citrulline recovery are evident between different groups receiving similar interventions. Some of this variability may be due to differences in the efficiency of arginine trapping by the Dowex column. The relative recovery across the dialysis membrane is dependent on the diffusion coefficient, which theoretically can be altered by ischemia and intracellular edema with decreased extracellular space and concentration of an endogenous substance. However, perfusion with L-NNA caused rapid and sustained suppression of labeled citrulline recovery during ischemia in our experimental system (Figure 2). This finding argues against the “pooling” of citrulline in the extracellular space with artificially concentrated recovery values. We have quantified the radial spread of labeled arginine from the probe with autoradiography in nonischemic rat brain.\textsuperscript{18} Our results indicated that the labeled compound spreads at a maximum diameter of 3 mm by 1 hour of perfusion at 1 \( \mu \)L/min. After 5 hours of perfusion, there is no further spread of label, suggesting that cellular uptake limits diffusion away from the probe site. Others\textsuperscript{41} have shown that labeled sucrose spreads cylindrically over a 1-mm-diameter by 14 minutes of dialysis perfusion. Finally, although probe insertion can cause disruption of the blood-brain barrier and an increase in extracellular space immediately around the probe,\textsuperscript{41} the volume of tissue sampled with our technique likely extends well beyond the injured volume.

In conclusion, this study demonstrates that FK506 pretreatment affords significant neuroprotection in the cortex and the caudoputamen complex in transient focal cerebral ischemia and reperfusion in the rat. Ischemia-evoked NO production during occlusion and acute reperfusion is not attenuated by FK506 at doses that provide neuroprotection. Although this neuroprotection may well involve several mechanisms, the drug does not act via inhibition of acute NO production and consequent early NO-mediated neurotoxicity.

\section*{Acknowledgments}

This work was supported by National Institutes of Health grants NS202020, NS33668, and NR03521. Dr Bhaward was supported in part by the American Heart Association Clinician-Scientist Award and the Richard S. Ross Clinician-Scientist Award from the Johns Hopkins University School of Medicine. The authors thank Dr Seiji Hashimoto, Exploratory Research Laboratories, Fujisawa Pharmaceutical Co, Tsukuba, Japan, for the gift of FK506.

\section*{References}

Neuronal nitric oxide synthase (NOS1) has been implicated in NMDA-induced neurotoxicity in cultures in vitro and in acute focal stroke in vivo. 1,2 Many pharmacological (ie, inhibitors) and genetic (ie, knockout mutants) strategies have been developed and used in studying the mechanisms of cell death via NOS1. Using a somewhat indirect but rather unique approach, Toung and colleagues now report that FK506, an immunosuppressant known to inhibit NOS1 activity by blocking its dephosphorylation in cultured neurons, is neuroprotective action of FK506 and other immunosuppressants, such as cyclosporin A, in brain injuries is the regulation of the mitochondrial permeability transition pore. 4,5 In view of recent euphoria about the mitochondrial role as a trigger in necrosis and apoptosis in acute stroke and in neurodegeneration, 6,7 pharmacological and therapeutic strategies are warranted to target the mitochondrial bioenergetic function and oxidative stress with immunosuppressants in stroke.

Pak H. Chan, PhD, Guest Editor
Departments of Neurosurgery, Neurology and Neurological Sciences
Stanford University Palo Alto, California

References

Neuroprotective FK506 Does Not Alter In Vivo Nitric Oxide Production During Ischemia and Early Reperfusion in Rats

Thomas J. Toung, Anish Bhardwaj, Valina L. Dawson, Ted M. Dawson, Richard J. Traystman and Patricia D. Hurn

*Stroke*. 1999;30:1279-1285
doi: 10.1161/01.STR.30.6.1279

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/30/6/1279

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/