

# Minocycline treatment in acute stroke

## An open-label, evaluator-blinded study



Y. Lampl, MD  
M. Boaz, PhD  
R. Gilad, MD  
M. Lorberboym, MD  
R. Dabby, MD  
A. Rapoport, MD  
M. Anca-  
Hershkowitz, MD  
M. Sadeh, MD

Address correspondence and  
reprint requests to Dr. Y.  
Lampl, Department of  
Neurology, Edith Wolfson  
Medical Center, Holon 58100,  
Israel  
y\_lampl@hotmail.com

### ABSTRACT

**Background:** Ischemic animal model studies have shown a neuroprotective effect of minocycline.

**Objective:** To analyze the effect of minocycline treatment in human acute ischemic stroke.

**Methods:** We performed an open-label, evaluator-blinded study. Minocycline at a dosage of 200 mg was administered orally for 5 days. The therapeutic window of time was 6 to 24 hours after onset of stroke. Data from NIH Stroke Scale (NIHSS), modified Rankin Scale (mRS), and Barthel Index (BI) were evaluated. The primary objective was to compare changes from baseline to day 90 in NIHSS in the minocycline group vs placebo.

**Results:** One hundred fifty-two patients were included in the study. Seventy-four patients received minocycline treatment, and 77 received placebo. NIHSS and mRS were significantly lower and BI scores were significantly higher in minocycline-treated patients. This pattern was already apparent on day 7 and day 30 of follow-up. Deaths, myocardial infarctions, recurrent strokes, and hemorrhagic transformations during follow-up did not differ by treatment group.

**Conclusions:** Patients with acute stroke had significantly better outcome with minocycline treatment compared with placebo. The findings suggest a potential benefit of minocycline in acute ischemic stroke. *Neurology*® 2007;69:1404-1410

### GLOSSARY

**ACE** = angiotensin-converting enzyme; **ACEI** = angiotensin-converting enzyme inhibitor; **BI** = Barthel Index; **mRS** = modified Rankin Scale; **NIHSS** = NIH Stroke Scale; **PUD** = peptic ulcer disease; **SU** = sulfonylurea.

Minocycline, a semisynthetic second generation derivative of tetracycline, was shown to have a clear beneficial neuroprotective effect in animal models of multiple sclerosis,<sup>1,2</sup> Parkinson disease,<sup>3,4</sup> Huntington disease,<sup>5,6</sup> and ALS.<sup>7,8</sup> Animal models provide promising evidence of minocycline's ability to improve outcomes in an animal stroke model. It has been shown that following stroke induction in the global brain ischemia model, pyramidal neuron survival increased from 10.5 to 77% after administration of minocycline.<sup>9</sup> This result was probably attributable to complete prevention of microglia ischemia-induced activation evidenced by the appearance of NADPH diaphorase reactive cells. A similar finding was observed in a rat model of transient focal cerebral ischemia with an infarct area reduction ranging between 63 and 76% and a concomitant inhibition of interleukin-1 $\beta$  converting enzyme, cyclo-oxygenase 2, and prostaglandin E expression.<sup>2,10</sup> Minocycline administration 48 hours post injury was found to significantly reduce infarct volume in the focal embolic cerebral ischemia model<sup>11</sup> and in reperfusion of the middle cerebral artery occlusion.<sup>12</sup>

The proposed mechanisms of minocycline include its anti-inflammatory effect,<sup>13</sup> reduction of microglial activation,<sup>9,14</sup> matrix metalloproteinase reduction,<sup>15</sup> nitric oxide production,<sup>16</sup> and inhibition of apoptotic cell death.<sup>17,18</sup> A protective effect of minocycline has been demonstrated in spinal cord culture against *N*-methyl-D-aspartate excitotoxicity.<sup>19</sup> Additionally, minocycline has a significant effect on the apoptotic cell death path-

From the Departments of Neurology (Y.L., R.G., R.D., A.R., M.A.-H., M.S.) and Nuclear Medicine (M.L.) and Epidemiology Unit (M.B.), Edith Wolfson Medical Center, Holon and Sackler Faculty of Medicine, Tel Aviv University, Israel.

*Disclosure:* The authors report no conflicts of interest.

way, including prevention of activated caspase 3 formation.<sup>6</sup> In neonatal stroke, minocycline treatment was found to provide early, but transient protection independent of the mitogen-activated protein kinase p38 pathway.<sup>20</sup>

In light of these findings, we performed an open-label clinical trial to investigate the efficacy of oral administration of 200 mg minocycline, which elsewhere had been proven safe, on the neurologic and functional outcomes in patients with acute ischemic stroke.

**METHODS** The study protocol was approved by our institution's ethics committee. Informed consent was obtained from all study participants. The study was designed as open-label, evaluator-blinded clinical trial. The primary objective of the study was to compare NIH Stroke Scale (NIHSS) scores on day 90 in subjects treated with 200 mg/day minocycline vs placebo. Secondary objectives of the study were to compare NIHSS scores on day 7 and day 30, as well as modified Rankin Scale (mRS) and Barthel Index (BI) scores on day 7, day 30, and day 90. Favorable outcome was defined as NIHSS 0 to 1 on day 90.

From May 2003 to June 2005, all patients with acute ischemic stroke were screened for eligibility. Inclusion criteria were as follows: 1) age >18; 2) NIHSS score >5; and 3) onset of stroke 6 to 24 hours prior to beginning of treatment. Patients who arrived within 0 to 6 hours post stroke were treated with other medications according to the best accepted medical treatment guidelines. Excluded were patients with 1) hemorrhagic stroke; 2) evidence of other disease of the CNS, including brain tumor, demyelinating disease, inflammatory disease of the CNS, craniotomy in the past, severe brain injuries, and idiopathic intracranial hypertension; 3) pre-existing neurologic disability; 4) known allergic response to tetracyclines; 5) acute or chronic renal failure; 6) pre-existing infectious disease requiring antibiotic therapy; and 7) swallowing difficulties. All patients were evaluated and treated according to the best accepted medical criteria and guidelines of treatment.<sup>21</sup>

Randomization to treatment assignment was performed using the 8th number of the subject's identity card. Assigned treatment was administered for 5 consecutive days. The neurologic deficits, global functional abilities, and the level of handicap were scored using the NIHSS findings,<sup>22</sup> BI,<sup>23</sup> and mRS score.<sup>24</sup> The NIHSS findings were categorized as complete or nearly complete improvement (0 to 1), mild (2 to 7), moderate (8 to 14), and severe (>15). The subjects were scored by a staff member blind to treatment assignment at baseline and on days 7, 30, and 90.

A sample size of  $n = 64$  per patient group had 80% power to detect a true, by-treatment difference of 2+ to 4 NIH points at day 90, assuming a two-sided  $\alpha$  of 0.05. The sample size was increased to preserve power in the event that assumptions were inconsistent with reality.

**Data analysis.** Analysis of data was carried out using SPSS statistical analysis software (SPSS Inc., Chicago, IL, 1999). For continuous variables such as age and NIHSS scores, de-

scriptive statistics were calculated and reported as means  $\pm$  SD. Normality of distribution of continuous variables was verified using the Kolmogorov-Smirnov test. All continuous variables were found to have distributions not significantly deviating from normal, so their means were compared by treatment group using the  $t$  test for independent samples. Categorical variables were compared by treatment group using the  $\chi^2$  test, exact as appropriate. The primary endpoint of the current study was the by-treatment difference in NIHSS score at day 90. Because a greater proportion of minocycline compared to placebo-treated patients received treatment with angiotensin-converting enzyme (ACE) inhibitors (ACEIs) and sulfonylurea (SU), and a smaller proportion had a history of peptic ulcer disease (PUD), these variables were entered as fixed factors along with treatment group in a general linear model of NIHSS at day 90. In this model, age and baseline NIHSS were entered as covariates. Additionally, a repeated measures analysis of NIHSS was carried out in which NIHSS scores on admission and on days 7, 30, and 90 comprised the endpoint, and treatment was entered as a fixed factor together with ACEI, SU, and PUD. Intermediate measures of NIHSS, mRS, and BI scores were compared by treatment group using the  $t$  test for independent variables. By-group differences in these exploratory endpoints were considered significant at  $p < 0.05$ . All tests were two tailed.

**RESULTS** Initially, 163 patients fulfilled the criteria for participation in the study. Two patients were excluded owing to known allergic reaction to tetracyclines, and eight patients refused to sign the consent form. One patient was excluded secondarily because of developing intracerebral bleeding during the period between inclusion and the beginning of treatment. In all, 152 patients (35.1% female; mean age  $66.7 \pm 11.11$ ) were finally included in the study. The time to treatment of the minocycline-treated group was an average of 12.64 hours: 28 patients received treatment between 6 and 9 hours, 20 between 10 and 12 hours, 7 between 13 and 15 hours, 5 between 16 and 18 hours, 6 between 19 and 21 hours, and 8 between 22 and 24 hours. The time to treatment of the placebo group was an average of 11.99 hours. No patient was lost to follow-up or dropped out owing to adverse events. Fourteen of the study patients (five minocycline-treated subjects and nine control subjects) died during follow-up.

Characteristics of the study population are shown in table 1. At baseline, by-treatment group differences in age, sex, or stroke etiology were not detected. As indicated, there were baseline differences in prescribed medications between the groups. Specifically, SU was observed more than four times as often in the treatment group than in the control group: 12 of 74 (16.2%) in the treatment group compared with 3 of 77 (3.9%) ( $p = 0.01$ ). ACEI was observed more frequently in the control group than in the treatment group: 40 of

**Table 1** Risk factors: Demographic and stroke data of both groups

	Minocycline-treated group, n (%)	Control group, n (%)
No. of patients	74 (47)	77 (51)
Gender	47 (63)	51 (66.2)
Age, y; mean $\pm$ SD	67.2 $\pm$ 11.1	66.2 $\pm$ 11.1
Previous stroke	17 (23.0)	17 (22.1)
Risk factors and other diseases		
Heart disease	22 (29.7)	24 (31.2)
Diabetes mellitus	27 (35.1)	25 (32.5)
Hypertension	45 (60.8)	50 (64.9)
Hyperlipidemia	18 (24.3)	27 (35.1)
Smoking	20 (27.0)	24 (31.2)
Renal failure	4 (5.5)	6 (7.7)
Chronic obstructive pulmonary disease	3 (4.7)	4 (5.1)
Malignancy (in past)	2 (2.8)	3 (3.8)
Psychotic disorder (in past)	2 (2.8)	3 (3.8)
Peptic ulcer disease (in past)	9 (11.7)	1 (1.4)
Hypothyroidism	2 (2.8)	4 (5.1)
Medications prior to events		
Antihypertensive		
ACEIs	25 (33.8)	40 (51.9)
$\beta$ -Blockers	25 (33.8)	27 (35.8)
Calcium channel blockers	15 (27.3)	18 (23.4)
Diuretics	10 (13.5)	8 (17.3)
Angiotensin receptor blockers	5 (6.8)	4 (5.1)
Antidiabetic		
Insulin	8 (17.8)	9 (11.6)
Sulfonylurea	12 (16.2)	3 (3.9)
Statins	24 (32.8)	24 (31.2)
Antiaggregation/anticoagulation		
Treatment (on admission)		
Aspirin (100–325 mg/d)	30 (40.6)	34 (44.1)
Clopidogrel (75 mg/d)	4 (5.4)	3 (3.9)
Warfarin	5 (6.8)	3 (3.9)
Etiology*		
Subtype 1 (atheromatosis)	29 (39.2)	31 (40.2)
Subtype 2 (embolus)	12 (16.2)	11 (14.3)
Subtype 3 (lacunar infarct)	22 (29.7)	22 (28.6)
Subtype 4 (other causes)	—	—
Subtype 5 (undetermined)	11 (14.9)	13 (16.9)
Location†		
Total anterior circulation	10 (13.5)	12 (15.6)
Partial anterior circulation	42 (56.8)	43 (55.9)
Lacunar syndrome	22 (29.7)	22 (8.5)

Differences among groups were found in pretreatment with ACEI ( $p = 0.02$ ), sulfonylurea ( $p = 0.01$ ), and peptic ulcer disease in the past ( $p = 0.01$ ).

\*Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.

†Oxfordshire Community Stroke Project (OCSP) classification.

ACEI = angiotensin-converting enzyme inhibitor.

**Table 2** NIH Stroke Scale (NIHSS), modified Rankin Scale (mRS), and Barthel Index (BI) scores by time of both groups

	Minocycline-treated group	Control group
NIHSS on admission	7.5 ± 3.2	7.6 ± 3.8
NIHSS on day 7, mean	6.5 ± 3.8	8.1 ± 4.4
NIHSS on day 30, mean	1.8 ± 2.1	7.1 ± 4.4
NIHSS on day 90, mean	1.6 ± 1.9	6.5 ± 3.8
mRS on admission, mean	2.8 ± 1.5	2.9 ± 1.4
mRS on day 7, mean	1.5 ± 1.4	3.1 ± 1.3
mRS on day 30, mean	1.1 ± 1.2	2.7 ± 1.3
mRS on day 90, mean	0.9 ± 1.1	2.1 ± 1.2
BI on admission, mean	70.0 ± 30.3	63.9 ± 29.6
BI on day 7, mean	85.9 ± 22.3	61.9 ± 30.8
BI on day 30, mean	90.6 ± 19.1	69.5 ± 26.6
BI on day 90, mean	94.9 ± 12.5	77.6 ± 24.0
Death		
Recurrent stroke or myocardial infarct during follow-up, n (%)	5 (6.8)	9 (11.1)
Hemorrhage transformation, n (%)	1 (1.4)	3 (3.9)

Differences ( $p < 0.0001$ ) for each of the tests were found in NIHSS days 7, 30, and 90, mRS days 7, 30, and 90, and BI days 7, 30, and 90.

77 (51.9%) vs 25 of 74 (33.8%) ( $p = 0.02$ ). More subjects in the placebo group than in the treatment group (1/74 patients [1.4%] vs 9/77 [11.7%]) had peptic ulcer in the past ( $p = 0.01$ ). No other variables differed significantly by group at baseline.

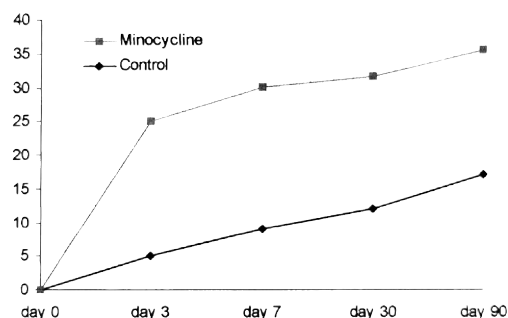
NIHSS findings were similar at baseline. At day 90, NIHSS scores were significantly lower in minocycline- vs placebo-treated patients ( $1.6 \pm 1.9$  vs  $6.5 \pm 3.8$ ,  $p < 0.0001$ ). This by-treatment difference was already apparent on day 7 ( $6.5 \pm 3.8$  vs  $8.1 \pm 4.4$ ,  $p < 0.0001$ ) and on day 30 ( $1.8 \pm 2.1$  vs  $7.1 \pm 4.4$ ,  $p < 0.0001$ ). In fact, by-treatment differences in NIHSS findings could be detected from day 1 and remained significant throughout follow-up (table 2; figures 1 and 2). By-treatment differences in BI could be detected from day 7 through day 90 (end of follow-up). Values for mRS differed at day 2 and remained significantly different throughout the remaining follow-up period (table 2; figure 3).

As there were some baseline differences between the groups, the primary analysis for 90-day NIHSS score was repeated using an analysis of covariance with covariates: patient age, peptic ulcer disease, ACEI, SU, and baseline NIHSS score. The difference between groups remained significant and, in fact, the difference in means increased slightly after adjusting for the effect of these covariates (details available from authors).

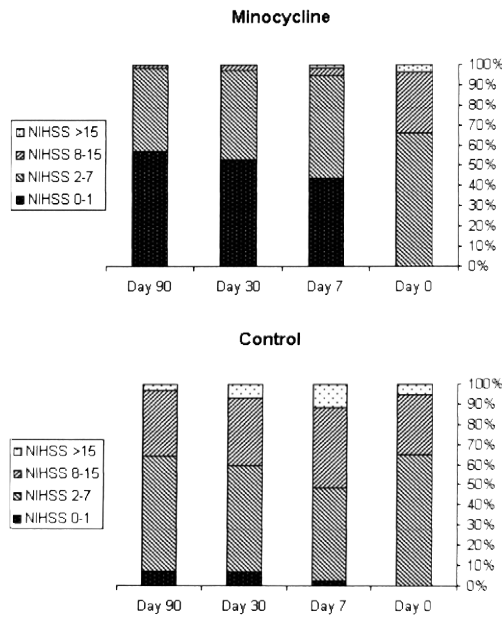
**DISCUSSION** The results of the current study suggest that administration of minocycline at the

acute stage of stroke is associated with better clinical outcome. The treatment benefit was observed in both primary and secondary endpoints. The study was designed to encompass a treatment period of 5 days with a therapeutic onset window of 24 hours. These decisions were based on the fact that the minocycline effect on ischemic brain tissue is at least partially dependent upon the inhibition mechanisms of the apoptotic pathway.<sup>17,18</sup> In vivo studies suggest that apoptotic cell death was observed 20 minutes after ischemia, peaking 24 to 48 hours later and persisting for 4 weeks.<sup>25</sup> Other studies have reported a direct relationship between the number of apoptotic cells and the duration of ischemia.<sup>26</sup> The reduction of apoptosis is

**Figure 1** Line graphs of both groups showing number of patients (on the y axis) with reduction of NIH Stroke Scale score by 4 grades or more (on x axis) with time



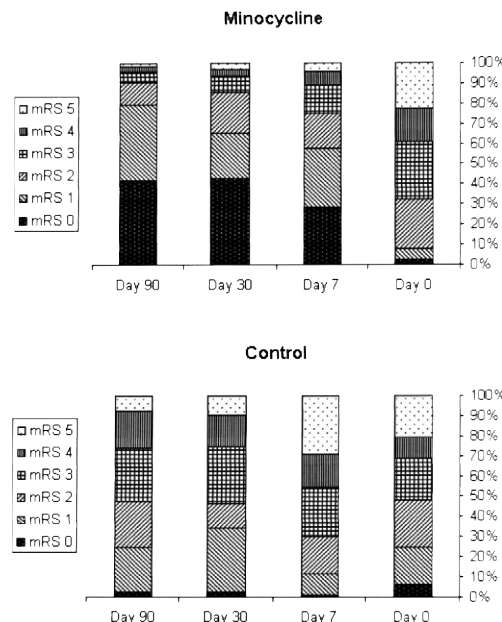
**Figure 2** NIH Stroke Scale (NIHSS) shift analysis of both groups



achieved through various mechanisms, probably on the mitochondrion level; it is based on the stabilization of mitochondrial membranes, cytochrome release into the cytosol, and activation of caspase 9 and 3.<sup>6,7,27,28</sup>

In this study, we failed to detect any difference between strokes with greater and smaller deficits or any association with location and/or etiology was detected. These results can be explained by the simultaneous induction of additional mecha-

**Figure 3** Modified Rankin Scale shift analysis of both groups



nisms by minocycline apart from the anti-apoptotic effect, namely, inhibition of microglial activation, induction of nitric oxide synthase,<sup>16</sup> and suppression of free radical production by depression of its release from the leukocytes.<sup>29</sup> Inhibition of the matrix metalloproteins, which induce the inflammatory process, and changes of the blood-brain barrier<sup>15</sup> are also shown to be related to minocycline activity. Other mechanisms are inhibition of T cell proliferation<sup>13</sup> and increasing tissue necrosis factor  $\alpha$ <sup>13</sup> and interleukin 6,<sup>13</sup> as well as chelating  $Ca^{2+}$ <sup>14</sup> and inhibition of p38 mitogen-activated protein kinase.<sup>20</sup> The varied characteristics of each of the pathway activities may be the basis for the broad spectrum of efficacy in this study as well as for the divergent efficacy findings in other nonstroke neurologic studies under minocycline administration.<sup>30</sup> The assumption that the improvement is ultimately due to the basic antibiotic effect of minocycline is not in agreement with the fact that there was no difference in serious adverse events among the two groups. Previous studies have already shown that the antibiotic effect has no influence on a better outcome of stroke.<sup>31</sup>

The favorable effects of minocycline in a therapeutic window of 72 hours can be due to the various minocycline mechanisms of action during stroke. It can be hypothesized that the anti-apoptotic effect, which has been shown in minocycline use,<sup>5,6</sup> will appear in a later poststroke stage after more than 48 hours. This assumption is based upon this effect being found in an animal model after 72 hours, whereas the nonapoptotic effect reached its maximal effect much earlier.<sup>32</sup>

Comparing the course of the control group with other control groups in previous studies, our control group seems to have had a worse outcome. This may be explained by the window of treatment time, which started 6 hours from the onset of stroke; therefore, patients with early spontaneous improvement and patients with transient ischemic attacks were completely excluded. The rapid treatment effect, regardless of the late and variable time to treatment, can be due to the various mechanisms of minocycline during the acute stage of stroke despite the primary effect on the apoptotic pathway.<sup>33-35</sup>

Apart from the promising results, the study has some limitations, which must be taken into consideration: 1) The study was designed as an open-label, evaluator-blinded study and confirmation by a double-blinded, controlled study is required; 2) the dosage of treatment medication, which was the proven safety dosage, might not

have been the optimal one; 3) patients received treatment orally—IV treatment may have been different and more effective; 4) the number of patients in each study group is relatively small—a much larger randomized study is necessary for confirmation of the results; and 5) the time window of treatment was between 6 and 24 hours post stroke—earlier treatment may have a different efficacy. As the sample of patients was small, it was not possible to isolate a subgroup of patients with a better outcome, according to different times of treatment onset.

*Received May 2, 2006. Accepted in final form April 27, 2007.*

## REFERENCES

1. Brundula V, Rewcastle NB, Mertz LM, Bernard CC, Yong VW. Targeting leukocyte MMPs and transmigration: minocycline as a potential therapy for multiple sclerosis. *Brain* 2002;125:1297–1308.
2. Popovic N, Schubart A, Goetz BD, Zhang SC, Lington C, Duncan ID. Inhibition of autoimmune encephalomyelitis by a tetracycline. *Ann Neurol* 2002;51:215–223.
3. Du Y, Ma Z, Lin S, et al. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proc Natl Acad Sci USA* 2001;98:14669–14674.
4. Wu DC, Jackson-Lewis V, Vila M, et al. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci* 2002;22:1763–1771.
5. Chen M, Ona VO, Li M, 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.
6. Wang X, Zhu S, Drozda M, et al. Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. *Proc Natl Acad Sci USA* 2003;100:10483–10487.
7. Zhu S, Stravrovskaya IG, Drozda M, et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature* 2002;417:74–78.
8. Tikka TM, Vartiainen NE, Goldsteins G, et al. Minocycline prevents neurotoxicity induced by cerebrospinal fluid from patients with motor neurone disease. *Brain* 2002;125:722–731.
9. Yrjanheikki J, Keinanen R, Pellikka M, Hokfelt T, Koistinaho J. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci USA* 1998;95:15769–15774.
10. Yrjanheikki J, Tikka T, Keinanen 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;96:13496–13500.
11. Wang CX, Yang T, Shuaib A. Effects of minocycline alone and in combination with mild hypothermia in embolic stroke. *Brain Res* 2000;963:327–329.
12. Xu L, Fagan SC, Waller JL, et al. Low dose intravenous minocycline is neuroprotective after middle cerebral artery occlusion-reperfusion in rats. *BMC Neurol* 2004;4:7.
13. Kloppenburg M, Brinkman BM, de Rooij-Dijk HH, et al. The tetracycline derivative minocycline differentially affects cytokine production by monocytes and T lymphocytes. *Antimicrob Agents Chemother* 1996;40:934–940.
14. Stirling DP, Khodarahmi K, Liu J, et al. Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. *J Neurosci* 2004;24:2182–2190.
15. Ryan ME, Usman A, Ramaurthy NS, Golub LM, Greenwald RA. Excessive matrix metalloproteinase activity in diabetes: inhibition by tetracycline analogues with zinc reactivity. *Curr Med Chem* 2001;8:305–316.
16. Amin AR, Attur MG, Thakker GD, et al. A novel mechanism of action of tetracyclines: effects on nitric oxide synthesis. *Proc Natl Acad Sci USA* 1996;93:14014–14019.
17. Lee SM, Yune TY, Kim SJ, et al. Minocycline reduces cell death and improves functional recovery after traumatic spinal cord injury in the rat. *J Neurotrauma* 2003;20:1017–1027.
18. Teng YD, Choi H, Onario RC, et al. Minocycline inhibits contusion-triggered mitochondrial cytochrome c release and mitigates functional deficits after spinal cord injury. *Proc Natl Acad Sci USA* 2004;101:3071–3076.
19. Tikka TM, Koistinaho JE. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol* 2001;166:7527–7533.
20. Lin S, Zhang Y, Dodel R, Farlow MR, Paul SM, Du Y. Minocycline blocks nitric oxide-induced neurotoxicity by inhibition p38 kinase in rat cerebellar granule neurons. *Neurosci Lett* 2001;315:61–64.
21. Adams H, Adams R, Del Zoppo G, Goldstein LB. Stroke Council of the American Heart Association; American Stroke Association. Guideline for the early management of patients with ischemic stroke: 2005 guidelines update a scientific statement from the Stroke Council of the American Heart Association/American Stroke Association. *Stroke* 2005;36:916–923.
22. Muir KW, Weir CJ, Murray GD, Povey C, Lees KR. Comparison of neurological scales and scoring systems for acute stroke prognosis. *Stroke* 1996;27:1817–1820.
23. Mahoney FI, Barthel DW. Functional evaluation: the Barthel Index. *Md State Med J* 1965;14:61–65.
24. Burn JP. Reliability of the modified Rankin Scale. *Stroke* 1992;23:438.
25. Li Y, Chopp M, Jiang N, Yao F, Zaloga C. Temporal profile of in situ DNA fragmentation after transient middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab* 1995;15:389–397.
26. Li Y, Chopp M, Jiang N, Zhang ZG, Zaloga C. Induction of DNA fragmentation after 110 to 120 minutes of focal cerebral ischemia in rats. *Stroke* 1995;26:1252–1258.
27. Wand J, Wei Q, Wang CY, Hill WD, Hess DC, Dong Z. Minocycline up-regulates Bcl-2 and protects against

- cell death in mitochondria. *J Biol Chem* 2004;279:1998–2054.
28. Scarabelli TM, Stephanou A, Pasini E, et al. Minocycline inhibits caspase activation and reactivation, increases the ratio of XIAP to smac/DIABLO, and reduces the mitochondrial leakage of cytochrome C and smac/DIABLO. *J Am Coll Cardiol* 2004;43:865–874.
  29. Gabler WL, Smith J, Tsukuda N. Comparison of doxycycline and a chemically modified tetracycline inhibition of leukocyte functions. *Res Commun Chem Pathol Pharmacol* 1992;78:151–160.
  30. Nessler S, Dodel R, Bittner A, et al. Effect of minocycline in experimental autoimmune encephalomyelitis. *Ann Neurol* 2002;52:689–690.
  31. Chamorro A, Horcajada JP, Obach V, et al. The early systemic prophylaxis of infection after stroke study: a randomized clinical trial. *Stroke* 2005;36:1495–1500.
  32. Hu X, Johansson IM, Brannstrom T, Olsson T, Wester P. Long-lasting neuronal apoptotic cell death in regions with severe ischemia after photothrombotic ring stroke in rats. *Acta Neuropathol (Berl)* 2002;104:462–470. Epub 2002 Jun 21.
  33. Tikka TM, Koistinaho JE. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglial. *J Immunol* 2001;166:7527–7533.
  34. Shimazawa M, Yamashima T, Agarwal N, Hara H. Neuroprotective effects of minocycline against in vitro and in vivo retinal ganglion cell damage. *Brain Res* 2005;1053:185–194.
  35. Song Y, Wei EQ, Zhang WP, et al. Minocycline protects PC 12 cells against NMDA-induced injury via inhibiting 5-lipoxygenase activation. *Brain Res* 2006; 1085:57–67.