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An open-label evaluator-blinded clinical study of minocycline neuroprotection in ischemic stroke: gender-dependent effect

Amiri-Nikpour Mohammad Reza, Nazarbaghi Surena, Hamdi-Holasou Milad, Rezaei Yousef.
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Objectives – Minocycline as an antibiotic has been found to have neuroprotective effect on neurodegenerative diseases. This study was aimed at determining the efficacy of minocycline adjunct to aspirin in improving neurological outcomes of ischemic stroke during 3-month follow-up. *Methods and materials* – In an open-label evaluator-blinded trial, 60 patients with ischemic stroke were allocated into two groups to receive either 200 mg of oral minocycline daily for 5 days during 6–24 h following onset of signs and symptoms, or not receiving any, as control; all patients also received 100 mg of aspirin daily. Clinical assessment at baseline and on days 30, 60, and 90 was performed using National Institutes of Health Stroke Scale (NIHSS) score. *Results* – Fifty-three patients (88.3%) completed the study. Females in the treatment and control groups were 53.8% and 51.9%, respectively ($P = 0.884$). Among all patients, NIHSS score was significantly lower in the minocycline-treated compared with control on day 90 (minocycline median 4, interquartile range 4–7, control median 7, interquartile range 5–8, $P = 0.031$). Among males, NIHSS was lower in minocycline-treated compared with controls on days 30, 60, and 90 ($P < 0.05$); however, females showed no significant differences at the same times compared with controls. No adverse outcomes including myocardial infarction, recurrent stroke, and mortality were observed in the both groups. *Conclusion* – Patients with ischemic stroke who received oral minocycline daily for 5 days had significantly better neurological outcomes on day 90 than controls. However, females showed no significant clinical improvement compared to males.

Mohammad Reza Amiri-Nikpour[1], Surena Nazarbaghi[1], Milad Hamdi-Holasou[1], Yousef Rezaei[2]

[1]Department of Neurology, Imam Khomeini Hospital, Urmia University of Medical Sciences, Urmia, Iran; [2]Seyyed-al-Shohada Heart Center, Urmia University of Medical Sciences, Urmia, Iran

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Yousef Rezaei, Seyyed-al-Shohada Heart Center, 17 Shahrivar Blvd, Urmia, Iran. 5718749441
Tel.: +98 912 623 1864
Fax: +98 441 237 5907
e-mail: yousefrezaei1986@gmail.com

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Introduction

Stroke is attributed to the central nervous system (CNS) focal injury caused by a vascular cause, including cerebral infarction and hemorrhages. Because of increase in population age, stroke burden will increase during the following 20 years (1), and based on the latest global burden of disease, up to 2010 year, it has been considered as the third most common cause of death and disability worldwide (2). As it has been previously proved, the great benefit for the management of stroke includes tissue plasminogen activator (TPA) intravenously or aspirin, warfarin, antiplatelet agents, cholesterol reduction, and surgical options (3).

Minocycline is a semisynthetic second generation of tetracycline derivative agent, which is used as an antimicrobial drug. In addition to its antimicrobial property, it has been recognized with an anti-inflammatory, anti-apoptotic, and neuroprotective ability in the experimental and clinical studies of some diseases, including stroke, multiple sclerosis, Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and spinal cord injury (4–10). Although its neuroprotective action has not been precisely defined, but some mechanisms have been

proposed. The main pathophysiological mechanisms of minocycline action consisted of the inhibition of cytochrome c release from mitochondria involved in cell death, the inhibition of caspase-1 and caspase-3 expressions, the inhibition of p38 MAPK (MAPK is a mitogen-activated protein kinase) activation in microglia, increased expression of 5-lipooxygenase, and the inhibition of poly (ADP-ribose) polymerase-1 (PARP-1) (11–14).

Given these points, we conducted an open-label evaluator-blinded clinical trial to investigate the effect of 200 mg of oral minocycline once daily lasting for 5 days adjunct to 100 mg of aspirin daily on neurological outcomes of ischemic stroke during 90-day follow-up period.

Methods and materials

This prospective study was designed as an open-label evaluator-blinded clinical trial to investigate the functional outcomes of ischemic stroke using National Institutes of Health Stroke Scale (NIHSS) score during 90-day follow-up in the patients who received either oral minocycline adjunct to aspirin, treatment group, or aspirin only, control group. The study was approved by the local ethics committee of our university, Urmia University of Medical Sciences, West-Azerbaijan province, Iran. All patients were also given written informed consent. The primary end point included the functional outcomes based on the NIHSS score on day 90 in the patients receiving minocycline compared with control. The secondary end point included changing NIHSS scores during follow-up on days 30 and 60.

Overall 60 patients diagnosed with acute ischemic stroke were randomized into two groups from January 2012 to December 2012. Inclusion criteria consisted of age over 18 years, signs and symptoms of cerebral stroke beginning at 6–24 h before undergoing treatment, NIHSS scores of >5, and the acute onset of focal neurological injury and a computed tomography (CT) imaging compatible with an acute ischemic stroke. Exclusion criteria included as follows: (i) hemorrhagic stroke diagnosed with CT scan; (ii) evidences of other CNS diseases, including brain tumor, demyelinating diseases, inflammatory diseases, a history of craniotomy or severe brain injuries, and idiopathic intracranial hypertension; (iii) an existence of neurological disabilities; (iv) allergic reactions to the tetracycline; (v) acute or chronic renal failure; (vi) infections needed antibiotic therapy before or during minocycline therapy; (vii) pregnancy; (viii) administration of thrombolysis therapy; and (ix) estrogen therapy.

All patients were randomized in a 1:1 ratio into two groups: (i) patients who received 200 mg of oral minocycline once daily during 6–24 h following onset of signs and symptoms for 5 days ($n = 30$); and (ii) patients who did not receive any as control group ($n = 30$). In addition, all of them received the standard basic therapy of acute ischemic stroke consisting of 100 mg of aspirin once daily. All participants received therapy within 9 h after arriving to the hospital. In addition, all allocated patients regardless of their group received rehabilitation therapy with similar features.

Baseline demographics including age, sex, and known risk factors for cerebrovascular diseases (a history of stroke, ischemic heart disease, diabetes mellitus, hypertension, dyslipidemia, smoking, and positive familial history) were collected upon admission to the emergency department. All patients were examined in terms of presenting signs and symptoms at admission. After allocation to the both groups, they were fully examined for evaluating their neurological deficits scored by NIHSS questionnaire at presentation time before receiving therapy and on days 30, 60, and 90. In addition, the NIHSS scores were divided into four groups based on the degree of favorable clinical outcomes: (i) 0–1 complete, (ii) 2–7 mild, (iii) 8–15 moderate, and (iv) >15 severe (15).

The categorical variables were analyzed using chi-squared test, and continuous ones analyzed using either *t*-test or Man–Whitney *U*-test as appropriate. The two-tailed *P*-values <0.05 were considered statistically significant. All analyses were performed using SPSS software, version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

From a total of 115 patients who were assessed for eligibility, 60 patients (52% recruiting rate) met inclusion criteria. Patients who were entered into the study were allocated into two groups. Seven patients (11.7%) were excluded from the study; therefore, 26 and 27 patients were analyzed in the minocycline-treated and control groups, respectively (Fig. 1).

The patients' mean age in the minocycline and control groups was not significantly different (65.23 ± 9 vs 66.52 ± 7.8 years, respectively, $P = 0.538$). Female sex in the both treatment and control groups was 53.8% and 51.9%, respectively ($P = 0.884$). All measured risk factors were comparable between groups, although there were

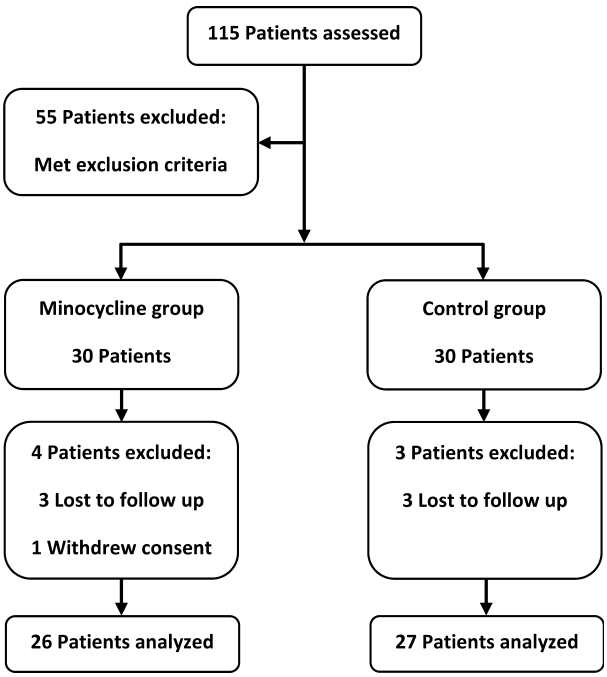

Figure 1. Study flow diagram.

no significant differences (Table 1). Regarding the drug histories, no significant differences were observed between groups except consuming antihypertensive plus antidiabetic agents, which was observed only among controls ($P = 0.005$). In addition, β-blocker use showed a trend toward control patients to take more than treatment group ($P = 0.074$). The location of stroke according to the imaging was reported as anterior or posterior circulation involvement; no differences existed between the both groups regarding the stroke location (Table 1, P = 0.407).

The NIHSS score at admission was not significantly different between the minocycline-treated and control groups (minocycline median 9, interquartile range 8–13, control median 9, interquartile range 8–13, $P = 0.501$). The NIHSS score was significantly lower in the minocycline-treated group compared with controls on day 90, as well (minocycline median 4, interquartile range 4–7, control median 7, interquartile range 5–8, $P = 0.031$). The amount of NIHSS score on days 30 and 60 was not significantly different between groups, although their values were lower in the treatment group compared with those of control (Table 2).

In addition, all patients were divided into two groups based on their sex, male or female. Among male participants, the NIHSS score at baseline was no significantly different between minocycline and control groups ($P = 0.098$); however, it was found to be significantly higher in control compared with those receiving minocycline on days 30, 60, and 90 (Table 2).

Table 1 Characteristics and risk factors of studied population based on groups

	Total ($n = 53$)	Minocycline ($n = 26$)	Control ($n = 27$)	*P*-value
Age (mean ± SD)	65.89 ± 8.35	65.23 ± 8.99	66.52 ± 7.80	0.538
Gender				0.884
Female	28 (52.8%)	14 (53.8%)	14 (51.9%)	
Male	25 (47.2%)	12 (46.2%)	13 (48.1%)	
Risk factors, No (%)				
Ischemic heart disease	17 (32.1%)	9 (34.6%)	8 (29.6%)	0.697
Hypertension	39 (73.6%)	18 (69.2%)	21 (77.8%)	0.480
Diabetes mellitus	26 (49.1%)	12 (46.2%)	14 (52%)	0.678
Dyslipidemia	23 (43.4%)	12 (46.2%)	11 (40.7%)	0.691
Previous stroke	10 (18.9%)	5 (19.2%)	5 (18.5%)	0.947
Familial history of stroke	5 (9.4%)	3 (11.5%)	2 (7.4%)	0.607
Smoking	19 (35.8%)	9 (34.6%)	10 (38.5%)	0.854
Drug histories, No (%)				
ACE-I	12 (22.6%)	6 (23.1%)	6 (22.2%)	0.941
ARB	7 (13.2%)	1 (3.8%)	6 (22.2%)	0.480
β-blockers	14 (26.4%)	4 (15.4%)	10 (37%)	0.074
Calcium channel blocker	6 (11.3%)	2 (7.7%)	4 (14.8%)	0.413
Diuretics	8 (15.1%)	4 (15.4%)	4 (14.8%)	0.954
Antidiabetics	18 (34%)	9 (34.6%)	9 (33.3%)	0.922
Statins	8 (15.1%)	2 (7.7%)	6 (22.2%)	0.140
Antihypertensive plus stains	6 (11.3)	2 (7.7)	4 (14.8)	0.413
Antihypertensive plus antidiabetics	7 (13.2)	0 (0)	7 (13.2)	0.005
Ischemia location				0.407
Anterior circulation	38 (71.7)	20 (76.9)	18 (66.7)	
Posterior circulation	15 (28.3)	6 (23.1)	9 (33.3)	

ACE-I, Angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 2 Clinical outcomes according to National Institutes of Health Stroke Scale (NIHSS) scoring during 3-month follow-up

	Minocycline	Control	*P*-value
NIHSS on admission (mean ± SD)			
Total	9 (8, 13)	9 (8, 12)	0.501
Female	12 (8, 14)	8 (7, 10)	0.014
Male	8.5 (7, 10)	9 (8, 13)	0.098
NIHSS on day 30 (mean ± SD)			
Total	6 (5, 9)	8 (6, 11)	0.221
Female	8.5 (5, 10)	7 (5, 8)	0.243
Male	5.5 (5, 7)	8 (7, 12)	0.007
NIHSS on day 60 (mean ± SD)			
Total	5.5 (4, 8)	7 (5, 9)	0.132
Female	7.5 (4, 10)	6.5 (5, 8)	0.443
Male	5 (3.5, 6)	8 (6, 10)	0.006
NIHSS on day 90 (mean ± SD)			
Total	4 (4, 7)	7 (5, 8)	0.031
Female	6.5 (4, 9)	6 (5, 7)	0.577
Male	4 (3.5, 4.5)	8 (6, 10)	0.001

Data are presented as median (25th and 75th percentiles).
NIHSS, National Institutes of Health Stroke Scale.

Comparing NIHSS score at baseline among female participants showed that its value was significantly higher in treatment group ($P = 0.014$). Moreover, no clinical improvement measured by NIHSS was observed among females during follow-up period (Table 2).

During 90-day follow-up, no adverse outcomes including myocardial infarction, recurrent stroke, and mortality were observed in the both groups.

Discussion

This study provided further evidence to demonstrate that the use of oral minocycline therapy adjunct to standard antiplatelet therapy of aspirin at presentation in patients diagnosed with acute ischemic stroke was effective for improving functional outcomes during 90-day follow-up. However, the efficacy of that regimen was shown only among male patients.

Despite being used as an antibiotic agent, minocycline is increasingly being found as a neuroprotective drug due to its high penetration into the brain-blood barrier and acting through mentioned pathways during neurodegenerative diseases (12, 16). In some experimental studies, it has been shown that minocycline led to neurological function improvement following ischemic stroke (11, 13, 17–19). Moreover, some clinical studies have been conducted among patients with acute ischemic stroke that those showed neuroprotective effect of oral minocycline (15, 20). Lampl et al. (15) showed, for the first time, that oral minocycline was effective for improving ischemic stroke outcome at follow-up compared with placebo. They gave patients either 200 mg of oral minocycline for 5 days or placebo and found significant efficacy to improve outcomes on days 7, 30, and 90. Padma and coworkers (20) also demonstrated neurological improvement on days 30 and 90 in patients receiving 200 mg of oral minocycline, as well. However, another recently published pilot study conducted by Kohler et al. (21) administered 100 mg of minocycline intravenously up to five doses in both ischemic and hemorrhagic strokes, and they revealed that it was not efficacious. Our findings are in consistent with those of oral minocycline results showing its promising effect on neurological outcomes following acute brain deficit.

All pathways involved in minocycline neuroprotective action have independent roles, and those mechanisms have different impacts on neurodegenerative diseases (15). Despite protection against some neurodegenerative diseases, minocycline has been found to have different impacts on brain damage following cerebral stroke in both sexes (14, 19, 22). Hoda et al. (19) revealed in their experimental study that minocycline protected adult females against thromboembolic stroke, while it showed no protection in aged males and females and ovariectomized females. In addition, Li et al. (14) found the lack of efficacy of minocycline for reducing ischemic stroke injuries in female mice. They concluded that minocycline protection in ischemic injuries was partly attributable to PARP-1 inhibition. They disclosed these neuroprotective effects might be because of mediating cell death in females by caspase activation not PARP-1 activation or of being male-dependent neuroprotection through inhibiting PARP-1 pathway. Minocycline did not also impact on ischemic injuries in PARP-1 null male mice, which revealed another rationale for role of PARP-1 inhibition mechanism (14).

The gender-dependent outcomes of ischemic stroke and hormonal effect on cell death after ischemia have been found in numerous studies. Ischemic stroke prognosis may be influenced by other mechanisms, which can be different in both sexes (22, 23). In addition, Weng et al. (24) in an experimental study demonstrated that using combined drugs with different mechanisms of effect may be of more benefit than either of them lonely, suggesting the utility of concomitant aiming at different pathological pathways. In the present study, we found, for the first time, that minocycline had no impact on improving neurological function following cerebral ischemia in females. Considering mentioned mechanisms and Weng findings, it is assumed that the lack of

minocycline neuroprotection in females may have been caused by either PARP-1 inhibiting or being involved another unknown pathophysiology.

The efficacy of 200 mg of oral minocycline has been shown in two previous clinical studies (15, 20), but it seems that we need further studies to detect the best effective dosage. In a dose-finding investigation (25), up to 10 mg/kg of intravenous minocycline has been found to be well-tolerated and safe adjunct to thrombolytic therapy, and in another study, it has been found that minocycline neuroprotection was dose-dependent, as well (26). Thus, it may be of great notion that clarifying the gender-dependent minocycline effect underlines the need for more randomized large-scale studies with focus on sex. Moreover, the therapeutic onset window of our study was 24 h, and it was according to the previous studies, in which it has been shown that cell death after ischemic stroke started at 30 min and peaked at 24–48 h and continued up to 4 weeks (27). Despite having therapeutic onset window similar to those of Lampl and Padma studies (15, 20), we believe that the time of treatment onset should be taken into consideration in future studies to confirm the gender-dependent effect of minocycline on ischemic stroke. Furthermore, minocycline was orally administered and it might contribute to different effect compared to that of intravenous injection. Given the two previous trials of oral minocycline (15, 20) and the only study of intravenous minocycline (21), it seems that comparative studies concerning the route of minocycline administration, either orally or intravenously, can be illustrative for identifying the most effective regimen.

Furthermore, not being placebo-controlled trail may be of a limitation of our study to show the exact effect of minocycline in the absence of placebo effect. Taking into account this notion, it has been found that the average amount of placebo effect is approximately 35% (28). However, a recent meta-analysis published has revealed that no superiority of placebo over no-treatment in clinical trials existed when the outcome had been an objective measurement (29). Due to lack of placebo-controlled study in terms of minocycline effect on ischemic stroke, we cannot precisely suppose that our results are relevant even without using placebo; therefore, more large-scale studies with regard to this notion will be of great importance.

Conclusion

Patients with acute ischemic stroke who received 200 mg of oral minocycline once daily for 5 days had significantly better neurological outcomes on day 90 than controls. However, female patients showed no significant clinical improvement compared with males. Further large-scale studies are required to take gender-dependency, dose-dependency, and the route of minocycline administration into consideration.

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None to declare.

Conflict of interest

All authors declare that there are no conflict of interests.

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