Minocycline Prevents IL-6 Increase after Acute Ischemic Stroke

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Abstract

Higher levels of the inflammatory biomarker interleukin-6 (IL-6) correlate with poor clinical outcome in acute ischemic stroke (AIS). Minocycline (MC) is a known anti-inflammatory agent; thus, the effect of MC on IL-6 in the first 24 h of AIS was investigated to determine potential anti-inflammatory activity. The Minocycline to Improve Neurologic Outcome in Stroke (MINOS) study was a non-randomized dose-escalation (3.0–10.0 mg/kg) trial of IV MC for AIS within 6 h of onset. Plasma IL-6 samples were collected prior to MC treatment at 1, 24, and 72 h and compared to those collected in a separate observational study of blood biomarkers in AIS. IL-6 levels were measured by commercially available ELISA kits. The lower limit of detection for IL-6 was 1 pg/ml. Sixty MINOS subjects and 29 non-MINOS subjects were enrolled, and there was no

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difference in baseline stroke severity. There was no significant difference in IL-6 level pre-MC treatment at 1, 24, or 72 h. However, the odds of a non-detectable IL-6 at 24 h in MINOS were 8.94 (95% CI 2.62–30.46) compared with non-MINOS subjects. It is likely that even low doses of MC have a potent systemic anti-inflammatory effect in AIS. Whether this results in improved outcome will be tested in a randomized clinical trial.

Keywords

Acute cerebral infarction; Emergency treatment of stroke; Inflammation; Interleukin-6; Biomarkers

Introduction

Post-stroke inflammation as characterized by elevated levels of inflammatory mediators, including interleukin-6 (IL-6), is correlated with both stroke severity and poor clinical outcome, prompting an interest in using agents with known anti-inflammatory activity in the acute phase of ischemic stroke (AIS) [1]. Minocycline (MC) is a potent anti-inflammatory agent with multiple mechanisms of action, a well-elucidated pharmacokinetic and safety profile, and compatibility with the only approved pharmacologic treatment for ischemic stroke (t-PA) [2, 3]. In this analysis, the relationship of intravenous MC administered to plasma IL-6 levels in AIS was investigated.

Methods

Subjects

Intravenous MC for AIS within 6 h of symptom onset was investigated in a non-randomized, dose-escalation trial (MINOS) [2]. Between June 2008 and October 2009, 60 subjects were enrolled into four dose tiers using the modified continual reassessment method (mCRM) from 3.0 to 10.0 mg/kg at the Medical College of Georgia (MCG), University of Kentucky and the Oregon Health Sciences University. MC was infused over 1 h every 12 h for 3 days, and blood samples for IL-6 analysis were collected pre-MC, 1 h following the first dose, 24 and 72 h. In a separate observational study of blood biomarkers in AIS, 29 subjects were enrolled between January 2006 and January 2008. Blood samples were collected at 24 h. Data from this population will be referred to as non-MINOS. The local Institutional Review Board approved these studies. Written informed consent was obtained from all participants or their legally authorized representative.

Laboratory Methods

Venous blood samples were collected in chilled, citrate-based, anticoagulated vacutainer tubes. They were centrifuged within 1 h at $2,000 \times g$ for 20 min at 4°C, and the plasma was removed, aliquoted, and frozen at −80°C. All measurements were performed in the biomarker laboratory by researchers blinded to the clinical data. IL-6 levels were measured using a commercially available ELISA kit from Thermo Scientific-Pierce (Rockford, IL). The lower limit of detection was 1 pg/ml. Intra- and inter-assay coefficients of variation were <10%. Measured values were graphically displayed alongside the published literature.

Statistical Analysis

All statistical analysis was performed using SAS 9.2. Due to having a large proportion of individuals with IL-6 levels at 24 h below the limit of detection, IL-6 levels were dichotomized into detectable or non-detectable levels. To examine whether an association between detectable IL-6 and MC existed at 24 h, logistic regression was used in controlling

for baseline National Institutes of Health Stroke Scale (NIHSS) and demographic variables. MC dose did not affect IL-6 level and was not included in the logistic regression analysis. Odds ratios and 95% confidence intervals were determined for having a non-detectable IL-6 level among those receiving MC compared to those not receiving MC. Statistical significance was assessed using an alpha level of 0.05.

Literature Search

A Medline search was conducted in August 2011 with the search terms *interleukin-6* and *acute stroke* present in the title or abstract. Results were limited to studies on humans and the English language. Studies without plasma IL-6 levels at 24-h post-stroke were excluded.

Results

There were 60 individuals in MINOS and 29 individuals in the comparison group. Using the mCRM, 11 MINOS subjects received 3.0 mg/kg, 4 received 4.5 mg/kg, 4 received 6.0 mg/ kg, and 41 received 10.0 mg/kg [2]. MINOS and non-MINOS demographics were similar for age, sex, hypertension, stroke subtype (lacunar vs. non-lacunar), and baseline stroke severity (NIHSS). The non-MINOS group had more African-Americans and diabetics, while MINOS had more subjects with atrial fibrillation (Table 1). There was no significant difference in IL-6 level pre-MC treatment at 1, 24, or 72 h (Fig. 1). However, the mean IL-6 at 24 h was 3.34 (SD = 10.18, median = 0) in MINOS and 12.82 (SD = 17.87, median = 10.10) in the non-MINOS group. A statistically significant association between MC and non-detectable IL-6 was found, with those given MC 8.94 times (95% CI 2.62–30.46) more likely to have a non-detectable IL-6 level at 24 h than those in the non-MINOS comparison group ($p = 0.0005$). IL-6 values with different doses were not statistically different from each other in post hoc tests (Fig. 2). At 90 days, the mean NIHSS in MINOS was 2.16 (SD = 3.27, median = 1.0) and 4.50 (SD = 4.99, median = 3.0) in the comparison group ($p = 0.1064$).

Twenty-one studies fit our systematic review criteria. Subsequently, only studies that reported data as median and IQR and that either did not dichotomize their results or only dichotomized IL-6 levels based on pre-defined good and poor outcomes were included, leaving eight studies for review [4–11]. When plotted against previous reports of IL-6 at 24 h after ischemic stroke, MINOS patients appeared markedly lower (Fig. 3).

Discussion

Following an acute stroke, serum markers of inflammation, including IL-6, are elevated [12]. Increased IL-6 is correlated with larger infarct volume, greater stroke severity, and worse clinical outcome [1]. In acute stroke, levels of IL-6 increase between admission and 24 h and remain elevated out to 1 week [14]. MC has potent anti-inflammatory properties and inhibits neutrophil, macrophage, and microglial cell activation [13]. No pharmacologic intervention has previously been shown to prevent this increase in IL-6 post-stroke. In MINOS, IL-6 levels were not statistically different between pre-treatment and 1, 24, and 72 h. However, compared with a separate cohort of ischemic stroke patients, MINOS patients had lower IL-6 at 24 h. Our data suggest that MC may blunt the normal rise in IL-6 after stroke, possibly reflecting anti-inflammatory activity.

Several limitations preclude more definitive conclusions about the impact of MC on IL-6. The study was not a randomized controlled trial; thus, despite subjects in the two groups being of similar age, sex, stroke severity, and stroke subtype, there were more African-Americans and diabetics in the non-MINOS group. However, there was no interaction between race or diabetic status and MC on IL-6, and we controlled for race and diabetes in

our analysis. Bias resulting from an unrecognized cofounder is also possible. Further, the non-MINOS group had a disproportionate population of diabetic and African-American patients compared to the MINOS group; however, this did not appear to affect IL-6. Also, IL-6 may be raised by concomitant infections or other inflammatory complications of stroke such as urinary tract infections, pneumonia, or deep vein thrombosis, but it is unlikely that these events occurred prior to 24 h [15]. Finally, although IL-6 has been correlated to the outcome, the clinical significance of lowering IL-6 levels remains to be fully elucidated.

In summary, IL-6 levels were lower at 24 h in patients from the MINOS study group compared to the non-MINOS patient group who did not receive MC. These findings support the anti-inflammatory activity of MC and potential in AIS. Due to its well-elucidated safety profile, compatibility with t-PA and anti-inflammatory activity, MC is an exciting prospect for use in AIS and supports a randomized controlled trial to investigate its effect on clinical outcome.

Acknowledgments

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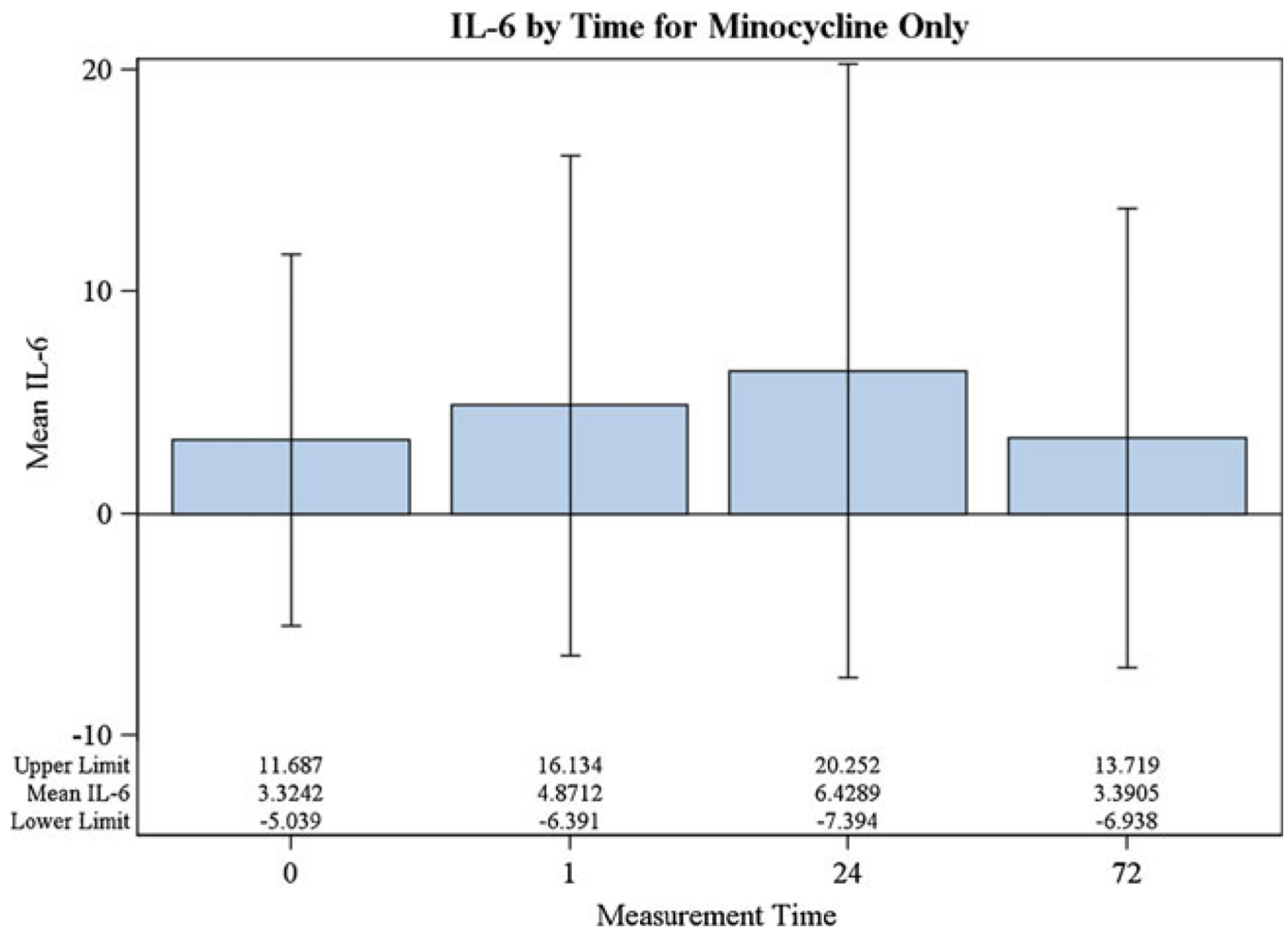
IL-6 by Time for Minocycline Only

	0	1	24	72
Upper Limit	11.687	16.134	20.252	13.719
Mean IL-6	3.3242	4.8712	6.4289	3.3905
Lower Limit	-5.039	-6.391	-7.394	-6.938

Measurement Time

Fig. 1.
IL-6 by measurement time in MINOS ($F_{(3,44.2)}$ = 2.75, p = 0.0541). No post hoc pairwise Bonferroni alpha-adjusted multiple comparison tests showed statistically significant differences
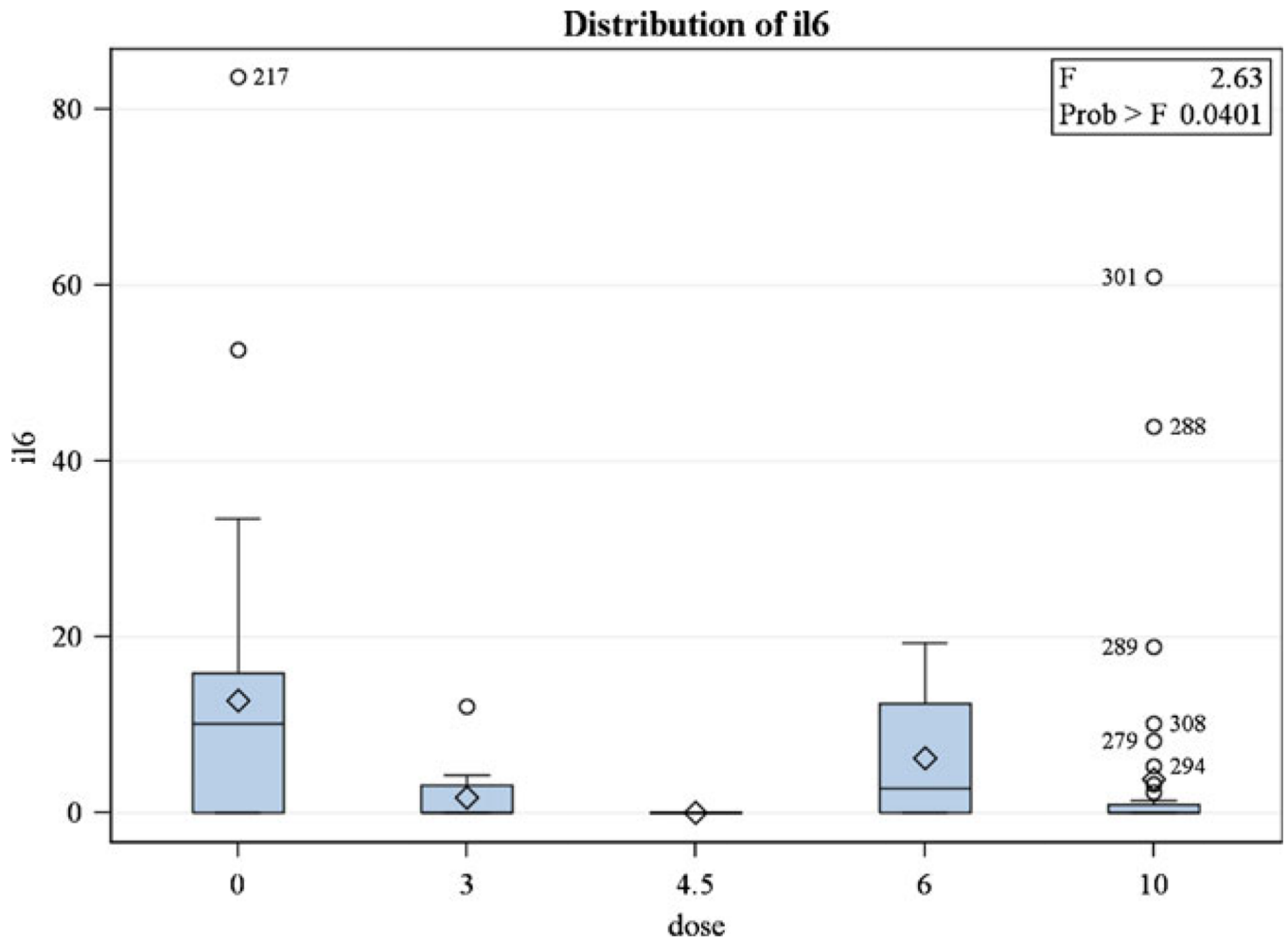
Fig. 2.
IL-6 by dose of minocycline at 24 h. The 0 dose is the non-MINOS comparison group
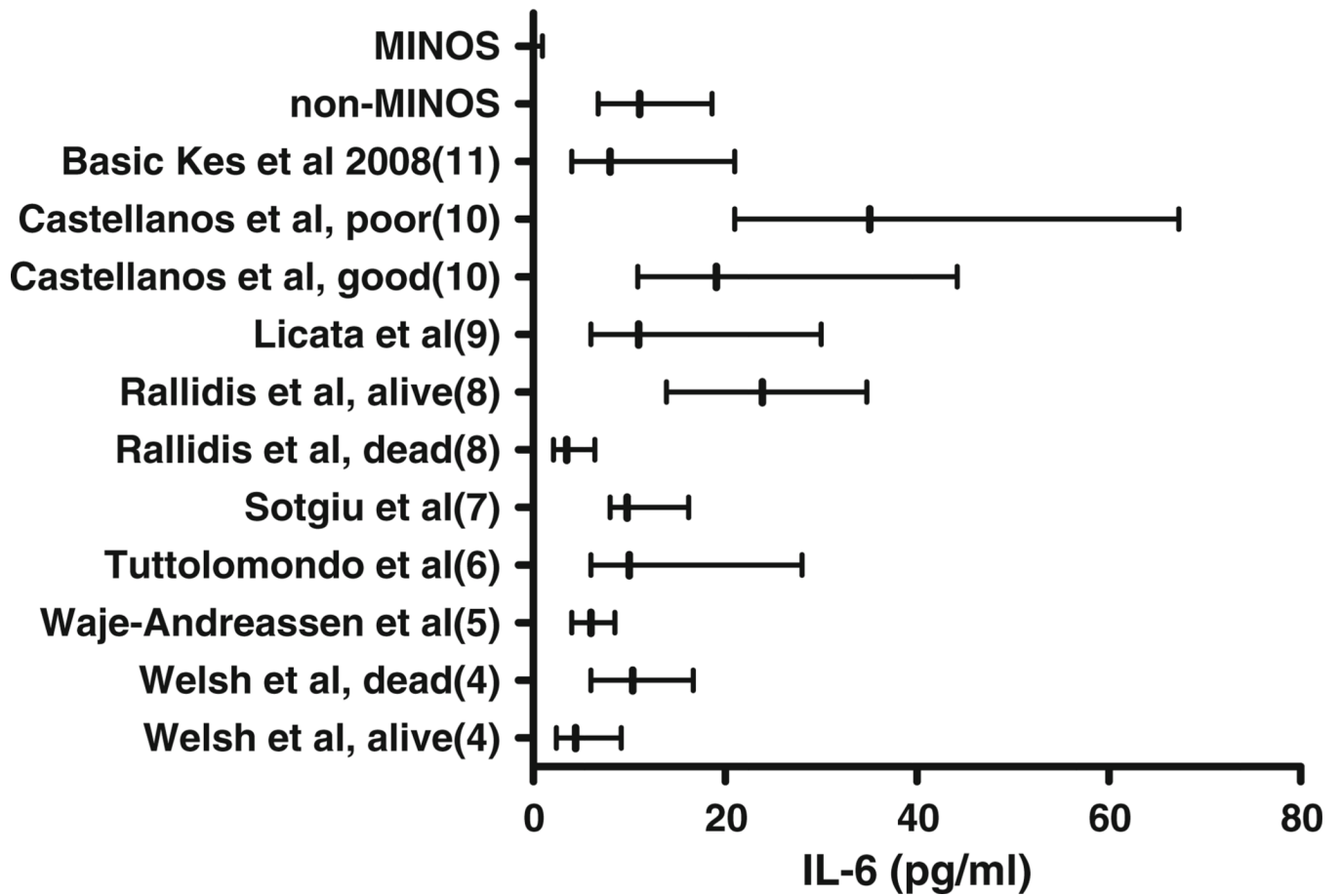
Fig. 3.
Levels of IL-6 within 24 h of onset of symptoms in acute ischemic stroke. Data displayed as median and IQR. Non-MINOS group was similar to other AIS populations, while the MINOS group visually was much lower than that of the published literature

Table 1

Descriptive statistics by MINOS and non-MINOS groups

Variable	Level	MINOS N=60		Non-MINOS N=29		p value
		N	%	N	%	
Race	Caucasian	50	83.3	16	55.2	0.0054
	African-American	9	15.0	13	44.8	
	Asian	1	1.7	0	0.0	
Gender	Male	32	53.3	15	51.7	1.0000
	Female	28	46.7	14	48.3	
tPA	No tPA	36	40.0	9	31.0	0.4867
	tPA	24	60.0	20	69.0	
Lacunar	Non-lacunar	52	86.7	22	75.9	0.2341
	Lacunar	8	13.3	7	24.1	
Hypertension	No	12	20.0	4	13.8	0.8415
	Yes	48	80.0	25	86.2	
Diabetes	No	44	73.3	13	44.8	0.0107
	Yes	16	26.7	16	55.7	
Atrial fibrillation	No	46	76.7	28	95.6	0.0311
	Yes	14	23.3	1	3.5	
Non-detectable IL-6 at 24 h	No	16	26.7	21	72.4	<0.0001
	Yes	44	73.3	8	27.6	
		Mean	SD	Mean	SD	
Age		65.04	13.72	64.45	13.93	0.8502
NIHSS at baseline		8.67 (median = 7.0)	5.83	9.52 (median = 8.0)	6.22	0.5564