Response to Letter by Lee Regarding Article, “Matrix Metalloproteinase-9 in an Exploratory Trial of Intravenous Minocycline for Acute Stroke”

Response:
We appreciate the interest in our study of the effect of minocycline on matrix metalloproteinase-9 (MMP9) in acute ischemic stroke.

Dr. Lee inquired as to our method of blood collection and sample analysis. For measurement of MMP9, venous blood was collected into chilled anticoagulated (citrate) vacutainer tubes. Samples were transported to the biomarker research laboratory on ice, centrifuged at 2000 g for 20 minutes at 4°C, the plasma removed, aliquoted, and frozen at −80°C. A 20-µL plasma sample (diluted 1:20 in running buffer) was directly loaded onto SDS-PAGE gels containing 1 mg/mL of gelatin and was separated under nonreducing conditions. This methodology of sample collection and analysis of plasma was identical to that in MINOS and our historical controls.

Dr. Lee also asked about our choice of zymography to measure MMP9. Zymography has several advantages. It allows us to evaluate the activity of all gelatinases, MMP9, and MMP-2, as well as intermediate forms of these enzymes and MMP9/NGAL complex as discussed below. We felt it was important to distinguish between changes in enzymatic activity as opposed to total level that could result from changes in the inactive form alone. Therefore, zymography was used to confirm the presence of the active isoform and to verify changes in MMP9 activity over time. We also measured the active form of MMP9 by ELISA in a subset of patients. In addition, a Western blot for active MMP9 was performed using an antibody that detects the active form. Although absolute values were different, the patterns were almost identical between zymography, ELISA, and Western analyses.

To investigate the cellular source of plasma MMP9 following stroke, we also measured MMP9/NGAL complex, a marker for MMP9 from neutrophils, with an ELISA that detects only the complex and not free forms of NGAL or MMP9 (R & D Systems). This study was supported by NIH/NINDS grants to D.C.H. (R01 NS055728) and S.C.F. (NS063965), Veteran Affairs Merit Review to S.C.F. and A.E., American Heart Association Established Investigator Award to A.E., and the Medical College of Georgia Intramural Grants Program Scientist Training Program to J.A.S.

Disclosures
None.

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