Apolipoprotein E Modifies Neurological Outcome by Affecting Cerebral Edema but not Hematoma Size after Intracerebral Hemorrhage in Humans

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Introduction: To address the mechanisms by which apoE polymorphism affects functional outcome after intracerebral hemorrhage in humans, we tested the hypothesis that the presence of the APOE4 allele results in amplified inflammatory responses and increased cerebral edema. *Methods:* We prospectively enrolled and collected data on 21 adult patients consecutively admitted to Duke University Hospital with supratentorial intracerebral hematoma including hemorrhage volume, midline shift, modified Rankin Score, Glasgow Outcome Score, and APOE genotype. Hemorrhage size (cm^3) and midline shift (mm), at the level of the thalamus, were measured by computed tomography within 36 hours of admission. Rankin and Glasgow Scores were determined at discharge. Student's *t*-test was used to analyze hemorrhage size, midline shift, and Glasgow Outcome Score and logistical regression were used to measure allele affect on modified Rankin Score. When analyzing modified Rankin Score, patients were grouped by favorable outcome (0-2) or unfavorable (3-6). *Results:* Out of 21 patients, 11 possessed at least 1 APOE4 allele (APOE4+). There was no difference in hemorrhage volume (25.8 *v* 38.3 mm for APOE4- *v* APOE4+, respectively) between the groups, but there was a significant difference in midline shift ($P = .04$, 0.7 *v* 4 mm). Functional outcomes were worse for the patients possessing at least 1 APOE4 allele ($P = .04$) *Conclusion:* The presence of APOE4 is associated with poor functional outcomes in humans after intracerebral hemorrhage. Our data suggest that the mechanism for this may be increased cerebral edema and not larger hematoma volume.

Intracerebral hemorrhage (ICH) is a devastating and relatively common disease affecting as many as 50,000 people annually in the United States alone. Unfortunately, despite advances in supportive care ICH remains associated with poor outcome, 30-day mortality approaches 40% to 50%.[1] Recent preclinical data suggest a number of inflammatory mechanisms that may play a crucial role in the development of cerebral edema and secondary neuronal injury.[2] Additionally, the importance of genetic influences on functional recovery remains an active area of investigation.[3]

In particular, apolipoprotein E polymorphism (APOE: gene; apoE protein) has been found to play an important role in modifying outcomes after ICH. The three common human isoforms of apoE, designated apoE2, apoE3, and apoE4, are encoded on chromosome 19, and differ by single cysteine to arginine interchanges at positions 112 and 158: E3($Cys_{112}Arg_{158}$); E4($Arg_{112}Arg_{158}$); E2($Arg_{112}Arg_{158}$).[4] Presence of the APOE4 allele has been demonstrated to

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Received June 18, 2008; revision received September 10, 2008; accepted September 12, 2008.

This study was made possible by funding through the Institute for the Study of Aging.

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1052-3057/$—see front matter

doi:10.1016/j.jstrokecerebrovasdis.2008.09.012

adversely effect outcomes in a number of acute brain injuries, including ICH.[5] Although a full understanding of the mechanism(s) by which apoE affects the CNS response to injury remains elusive, there is increasing evidence supporting a role for apoE in down-regulating endogenous brain inflammatory responses in an isoform-specific fashion. This is consistent with the known immunomodulatory properties of apoE,[6-8] as well as with recent observations demonstrating isoform-specific effects of apoE in modifying secretion of inflammatory mediators and brain edema following closed head injury.[9]

Neuroinflammatory responses play an important role following ICH, and the release of inflammatory cytokines increases blood brain barrier permeability, cerebral edema, and secondary neuronal injury. There is mounting preclinical data that cerebral edema, as a result of this inflammatory response, is attributable to a number of cellular mechanisms that likely conclude as a final common pathway to neuronal injury.[2] Thus, the isoform-specific effect of apoE on neuroinflammation may be particularly relevant in modifying outcomes after ICH, and several clinical studies have implicated the presence of APOE4 with poor outcome in this setting.[10,11] Therefore, to further define the clinical relevance and possible mechanisms of these genotype effects, we evaluated a commonly accepted radiographic surrogate of cerebral edema to investigate whether endogenous APOE genotype modifies the CNS inflammatory response after ICH with subsequent alteration in functional neurological outcome.

Methods

After Institutional Review Board approval and obtaining informed consent, consecutive adult patients with computed tomography (CT)-proven supratentorial ICH admitted to the Duke University Hospital Neuroscience Intensive Care Unit (NICU) were prospectively enrolled between January, 2005 and December, 2006. Exclusion criteria included patients under the age of 18 years, known pregnancy, admitting diagnosis of subarachnoid hemorrhage, infratentorial hemorrhage, and known traumatic etiology. Upon entry into the study, blood samples were obtained for APOE genotyping, demographics were recorded, and Glasgow Coma Score (GCS) and ICH Score[12] were tabulated. During the first 36 hours after admission, CT scans were obtained and evaluated for hemorrhage volume (cm^3), midline shift (MLS, mm), an accepted surrogate for cerebral edema, and presence of intraventricular hemorrhage (IVH); additionally, the latency from ictus to the study scan was recorded. Upon discharge from the hospital, modified Rankin score (mRS), Glasgow Outcome Score (GOS), hospital length of stay (LOS), and NICU LOS were recorded by a single observer blinded to genotyping. At analysis, patients were grouped by the presence of an APOE4 allele (ie, patients with an APOE4 allele [APOE4+] *v* patients without an APOE4 allele [APOE4-].

Determination of Radiographic Outcomes

All patients enrolled into the study were diagnosed with CT-proven ICH. The imaging scan used for the study protocol was the next subsequent scan that occurred within 36 hours after admission. A blinded neuroradiologist assessed CT scans in the following manner: On the CT slice with the largest area of ICH, the largest diameter (A) of the hematoma was measured in centimeters. The dimension of the hemorrhage perpendicular to the largest diameter represented the second diameter (B) in centimeters. The height of the hematoma was calculated by multiplying the number of slices involved by the slice thickness, providing the third diameter (C). The 3 diameters were multiplied and then divided by 2 (AxBxC/2) to obtain the volume of ICH in cubic centimeters.[13] For the purpose of determination of (C) diameter, the first and last slices where hematoma is first and last noted were not counted. Additionally, intraventricular hemorrhage (IVH) was defined as an intraventricular hyperdense image not attributable to calcification or the choroid plexus. MLS of the septum pellucidum were measured in millimeters by a blinded neuroradiologist and corrected for magnification by using the scale provided on each CT image. MLS was calculated as the distance from the center of the anterior horns of the lateral ventricles on the CT slice containing the third ventricle to a perpendicular line connecting the anterior and posterior insertions of the falx cerebri. Because individual measurements were recorded in millimeters, only MLS of greater than 2 mm were considered to be significant and recorded.

Human apoE Genotyping

A polymerase chain reaction (PCR)-based assay was used to amplify a short polymorphic region residing within coding sequences of the human APOE gene. Each amplification reaction was performed using 20 to 100 ng of genomic DNA, 1.0 pmol/mL of each primer, 10% dimethylsulfoxide, 1.5 mM $MgCl_2$, 200 mM of each dNTP, 0.05 U/mL Taq DNA polymerase (Promega, Madison, Wis) and supplied buffer in a final volume of 15 mL. The forward primer was 5′ TAAGCTTGGCACGGCT GTCCAAGGA 3′ and reverse primer was 5′ACAGAA TTCGCCCCGGCCTGGTACACTGCCA 3′. An initial denaturation at 94°C for 5 minutes was followed by 35 cycles of annealing at 65°C for 0.5 minutes, extension at 70°C for 45 seconds, denaturation at 94°C for 0.5 minutes, and a final extension at 70°C for 10 minutes. Following amplification a 5 μL mixture composed of 2 to 5 U of the restriction enzyme Hha I (Promega), 2.5 μL of Hha I 10× buffer, and dH2O to a final volume of 5 μL was added directly to each well and the reaction incubated for 1 to 2 hours at 37°C. Resultant DNA fragments were