The Immune Response to Intracerebral Hemorrhage and Effects of Heme Products.

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Intracerebral hemorrhage (ICH) is a stroke subtype with high rates of mortality and morbidity, with only 32% of patients surviving one year. Both heme components and the immune system have been implicated as secondary injury mechanisms after ICH. Because heme and related compounds have immunomodulatory effects it is logical to ask whether there is an interaction between these compounds and the immune system after ICH. In this report we have developed a mouse model of ICH and have utilized flow cytometry to quantitatively profile immune cell populations that infiltrate the brain at 1 and 4 days post-ICH. We have also examined the effects the heme products bilirubin and bilirubin oxidation products (BOXes) on leukocytes *in vitro* and in response to ICH. BOXes are heme-derived vasoactive molecules and they were found in the brain after ICH. Mice displayed typical markers of brain injury after ICH, including increased brain water content, apoptosis and decreased rotarod scores, a measure of motor function. At 4 d, ICH mice presented with a 2.4-fold increase in total blood derived immune cells, a 1.9-fold increase in cells of the lymphoid lineage/macrophages, a 3.4-fold increase in neutrophils and a 1.7-fold increase in CD4 T cells ($p \leq 0.05$ for all groups), compared to saline control mice; only neutrophils were elevated at 1 d. *In vitro,* bilirubin and BOXes inhibited PMA-induced superoxide production by peripheral leukocytes. *In vivo* we found differences in reactive astrocytosis between mice receiving blood and those receiving blood plus BOXes. Finally, BOXes did not affect total blood derived cells appearing in the brain after ICH. Our data suggest that CNS infiltrating inflammatory cells have a role in the response to ICH and that heme products may modulate this immune response in a complex way.

Supported by: NIH R01NS050569 and NIH T35DK060444