Haptoglobin Genotypes as Confounding Variables in Clinical Trials of Aneurysmal Subarachnoid Hemorrhage

Kevin W. Hatton MD, FCCM*[1], Alexandria Early BS[2] and Peter Morris MD[3]

[1]Division of Critical Care Medicine, Department of Anesthesiology, USA

[2]Departments of Anatomy and Neurobiology, USA

[3]Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, USA

*Corresponding author: Kevin W. Hatton, MD, FCCM, Division of Critical Care Medicine, Department of Anesthesiology, Lexington, USA

ARTICLE INFO

ABSTRACT

Aneurysmal Subarachnoid Hemorrhage (aSAH) is a severe form of hemorrhagic stroke resulting from rupture of an intracranial aneurysm. aSAH results in significant primary and secondary neurologic injuries, including hydrocephalus, Delayed Cerebral Ischemia and Cerebral Vasospasm (CV) that leads to chronic neurologic disability. Haptoglobin (Hp) is a naturally-occurring protein that binds to free hemoglobin (Hb) to facilitate its macrophage-based metabolism. In humans, there are three Hp genotypes (Hp1-1, Hp1-2, and Hp2-2) that lead to Hp molecules with different sizes, conformations, and Hb-binding affinities. The prevalence of these three Hp genotypes is variable within different populations. The greatest prevalence of the Hp2-2 genotype exists in native populations from India and Southeast Asia. In observational studies, this Hp2-2 genotype is associated with a two-fold increased risk of CV compared to the Hp1-1 and the Hp1-2 genotypes. To date, no pre-clinical or clinical trials have included this important confounding variable in power analyses, randomization schemes, or post-hoc data analysis.

In addition, since the Hp1-2 and the Hp2-2 genotypes exist only in humans, basic science research is routinely conducted only in animals that have only the Hp1-1 genotype, limiting the reliability of these results in humans. Future basic science studies of CV in aSAH should incorporate the different Hp genotypes into animal models, using previously-created transgenic models. In addition, future pre-clinical and clinical trials should also account for the effects of the different Hp genotypes in trial design and randomization schemes to minimize the effect of this confounding variable.

Keywords: Subarachnoid Hemorrhage; Intracranial Aneurysm; Cerebral Vasospasm; Haptoglobin

Introduction

Aneurysmal Subarachnoid Hemorrhage (aSAH) is a severe form of hemorrhagic stroke, affecting approximately 30,000 people in the United States and approximately 500,000 people worldwide each year [1]. Although aSAH occurs primarily in healthy patients aged 30-64 and presents suddenly and without warning, as an acute, severe headache with varying neurologic symptoms from minimal neck pain to seizures and coma [2]. Sadly, aSAH is frequently fatal and, for those patients who do survive, a significant proportion will have permanent neurologic disability [3,4]. To date, few treatments have improved long term neurologic outcomes or reduced neurologic disability in patients with aSAHn [5].

aSAH occurs when an intracranial aneurysm ruptures, releasing oxygenated arterial blood into the subarachnoid space where it mixes with pre-existing Cerebrospinal Fluid (CSF). Arterial blood in this space results in a rapid increase in Intracranial Pressure (ICP), severe headache and neck pain. Continued hemorrhage

into this space with resultant increases in ICP may result in additional neurologic signs and symptoms, including focal or generalized deficits, seen as nerve palsies, paralysis, or even loss of consciousness. Uncontrolled arterial bleeding may lead to catastrophic elevations in ICP, brain and brainstem herniation, and even death. For many patients, the initial bleeding stops, for unknown reasons, prior to catastrophic injury. If this happens and emergency help is sought, aggressive medical and surgical care can be provided at specialized hospitals, including the placement of CSF drains, the monitoring and treatment of elevated ICP, the obliteration of bleeding aneurysms using endovascular or surgical techniques, and monitoring for secondary neurologic injuries.

Complications of aSAH

For those patients who survive the initial neurologic injury and are stabilized in a neurocritical care unit, the focus of ongoing care turns to the treatment of secondary neurologic injuries, including chronic hydrocephalus, Delayed Cerebral Ischemia (DCI), and Cerebral Vasospasm (CV) [6,7]. Chronic hydrocephalus occurs when native CSF conduction and elimination system is disrupted by the blood mixed with CSF. These patients require early CSF drainage through a ventriculostomy and placement of an Extraventricular Drain (EVD). Some of these patients will also require a Ventriculoperitoneal Shunt (VPS) for chronic drainage of CSF. DCI occurs when patients have additional or new neurologic deficits that cannot be explained by other medical conditions, typically delayed days or weeks after the primary injury, without radiographic evidence of additional injury. CV is a spastic narrowing of intracranial arterial vessels, limiting blood flow to regions of the brain that typically occurs 3-10 days after aneurysm rupture. CV may affect vessels either near or far away from the ruptured aneurysms and may result in cerebral infarction if not rapidly diagnosed and treated with restoration of normal blood flow through super-selective injection of intra-arterial vasodilators, such as verapamil, papaverine or nicardipine using digital subtraction angiography. CV is an important cause of long-term neurologic disability and a significant proportion of the translational and clinical research in aSAH has aimed at improving our understanding of the pathophysiology, diagnosis and treatment of this catastrophic complication. To date, despite pre-clinical successes, no clinical trials have significantly improved long-term neurologic outcomes in patients with aSAH and CV [8-11].

One possible reason for these failed clinical trials may be the effect of an under-appreciated confounding variable, existing in humans but not animals, thereby effecting the clinical data significantly more than the pre-clinical data. In this review, we will describe the possibility that haptoglobin genotype may represent an important confounding variable in pre-clinical and clinical trials of CV in aSAH.

Haptoglobin Structure and Function

Haptoglobin (Hp) is a naturally-occurring, α-2 sialoglycoprotein produced primarily in the liver that binds free hemoglobin (Hb) molecules in blood, facilitating their metabolism, and preventing kidney injury and systemic iron loss [12]. Hp is an acute phase reactant whose production in increased by circulating proinflammatory cytokines, namely IL-1β, IL-6, and TNF-α [12]. Normal Hp molecules are a tetramer of 2 polypeptide chains created from 2 copies of the lighter Hpα polypeptide chain and 2 copies of the heavier Hpβ polypeptide chain [13]. The Hpα polypeptide chains are linked together via a single disulfide bridge. Each of these internal chains binds a single external Hpβ polypeptide chain. Free Hb binds to these heavier Hbβ polypeptide chain units, forming a Hp-Hb complex that undergoes macrophage endocytosis, mediated by the macrophage CD163 cell-surface receptor, before enzymatic degradation within intracellular lysosomes [13]. Macrophage CD163, a Scavenger Receptor Cysteine-Rich (SRCR) protein, also functions as part of the inflammatory cascade and as a pattern recognition receptor for invading pathogens [14,15].

In humans, there are three major Hp genotypes, differentiated by the genetic makeup of the Hpα amino acid sequence located at chromosome 16q22. While several different sequences exist, the predominant sequences, known as Hpa1 and Hpa2, code for small polypeptide chains with the abnormal Hpa2 sequence being long, containing a sequence identical to the wild-type Hpa1 code, followed by a second, near-duplication of that same sequence [13,16]. This duplicated sequence adds a second disulfide binding site to the internal Hpa2 polypeptide chain, creating opportunities for larger and abnormally-shaped Hp molecules with 3, 4 or even 5 Hb binding sites [12,13]. The different Hpα gene sequences allow for the three major Hp genotypes: Hp1-1 (homozygous Hpa1), Hp1-2 (heterozygous Hpa1-Hpa2), and Hp2-2 (homozygous Hpa2). Importantly, non-human animal species such as chimpanzee, rabbits, and mice have only the wild-type Hpa1 sequence and, therefore, present only the Hp1-1 genotype natively.

Haptoglobin Genotypes Worldwide

These three Hp genotypes exist in endemic populations at different frequencies based on their geographic and ethnic origin. For example, in native Indians and Australian aborigines, Hp2-2 is the most common genotype, occurring in 84% and 66% of these populations, respectively [17,18]. Likewise, the Hp2-2 genotype is the most common genotype in Southeast Asia, including Thailand, China, Taiwan, Korea and Japan. On the other hand, in Sub-Saharan Africa, the Hp1-1 is the most common genotype. Europe, South American and North America are a blend of these different geographic origins with the Hp1-2 genotype occurring most frequently with significant variation in the ratio between the Hp1-1, Hp1-2, and Hp2-2 genotypes among and within the individual countries.

Haptoglobin Genotypes in aSAH

Six small observational studies and 1 meta-analysis of those studies have shown that Hp genotype impacts the clinical outcomes in aSAH [19-25]. In general, these studies are all small, single-centered observational studies with poorly-defined or non-standard definitions of clinical endpoints of CV, DCI, and neurologic disability. These studies also report a significant heterogeneity in the prevalence of the haptoglobin genotypes within the study populations. For example, the Hp2-2 prevalence ranged from

4% to 52% of the studied populations with a mean prevalence of approximately 31%. Nonetheless, these studies all suggest that the Hp2-2 genotype is significantly associated with greater CV and worse clinical outcome. In fact, the published meta-analysis reported an Odds Ratio of 2.37 (95% CI=1.12-5.04) for CV in patients with the Hp2-2 genotype compared to the Hp1-1 genotype, an Odds Ratio of 1.73 (95% CI=1.02-2.93) for CV in patients with the Hp2-2 genotype compared to the Hp1-2 genotype, and an Odds Ration of 2.07 (95% CI=1.26-3.41) with the Hp2-2 genotype compared to both the Hp1-1 and the Hp2-12 genotypes [25].

Haptoglobin as Confounding Variable

Despite our growing understanding of the effect of the Hp2-2 genotype on CV in aSAH, no clinical studies have included Hp genotype differences in their power analyses or have risk-stratified for Hp genotype in their randomization schema. It is possible that the results of previously-published clinical trials have been impacted by this important confounding variable through an unexpected effect on outcome by a disproportionate recruitment of Hp genotypes into either the intervention or control arms of the trials [26,27]. Because data is not available on Hp genotypes characteristics in these trials, it is unclear whether the magnitude of this effect would have significantly altered the final conclusions [28]. Also, as previously mentioned, different Hp genotypes exist only in humans. Although genetically-modified mice with the Hp2-2 genotype have been recently developed, animals frequently used for basic science and pre-clinical trials in aSAH have historically only had the native Hp1-1 genotype [29]. Therefore, our understanding of the development of CV in aSAH exists primarily from the study of Hp1-1 animals. In addition, pre-clinical trials of newly-developed therapies have, historically, only utilized Hp1-1 animals. It is likely that treatments developed under these conditions will not (and have not) reflected therapies that will ultimately be successful in human disease.

Conclusion

Haptoglobin is an important, naturally-occurring, acute phase reactant that binds free Hb molecules and promotes normal Hb metabolism. The Hp genotype significantly effects the rate of CV after aSAH, leading to more than twice as much CV compared to other Hp genotypes. To date, no clinical studies have utilized Hp genotype differences in power analyses or randomization schemes to protect against this potential confounding variable. Future pre-clinical and clinical trials should consider the Hp genotype effect and the prevalence of the different Hp genotypes in their population, noting that multicenter trials may have significant variation in the Hp genotype prevalence, between their enrolling sites. If possible, previously-published studies should consider re-analysis of their data based on Hp genotypes within their treatment and control groups.

Acknowledgement

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