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The Role of Hemoglobin Oxidation Products in Triggering Inflammatory Response upon Intraventricular Hemorrhage in Premature Infants

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Introduction: Intraventricular hemorrhage (IVH) is a frequent complication of prematurity that is associated with high neonatal mortality and morbidity. IVH is accompanied by red blood cell (RBC) lysis, hemoglobin (Hb) oxidation, and sterile inflammation. Recent studies showed extracellular Hb, methemoglobin (metHb) and heme to be involved in inflammation following IVH. They also highlighted the role of Hb oxidation and heme release in inflammation after the onset of IVH.

Aims: Our goal was to perform a qualitative and quantitative analysis of Hb content of human cerebrospinal fluid (CSF) collected from premature infants after the onset of IVH. We also wanted to investigate the pro-oxidant and pro-inflammatory effects of Hb forms on human brain microvascular endothelial cells.

Methods: We collected cerebrospinal fluid (N=20) from premature infants with grade III IVH at different time points after the onset of IVH. Levels of Hb, metHb, and ferrylHb were determined by analysis of the visible spectra of CSF samples. Oxidative Hb crosslinking was assessed by Western blot. Concentrations of soluble adhesion molecules VCAM-1, and ICAM-1 and the pro-inflammatory cytokine IL-8 in CSF were determined by ELISA. Our in vitro experiments were performed on human brain microvascular endothelial cells (HBECS).

Results: Levels of Hb, metHb, total heme and free heme were the highest in CSF samples obtained between days 0-20 after the onset of IVH and were mostly non-detectable in CSF collected between days 41-60 of post-IVH. Besides Hb monomers, we detected crosslinked Hb dimers and tetramers in post-IVH CSF samples obtained in days 0-20 and 21-40, but only Hb tetramers were detected in CSF samples obtained after 41-60 days. VCAM-1 and IL-8 levels were higher in CSF samples obtained between days 0-20 than in CSF samples collected between days 41-60 of post-IVH. Applying free heme and oxidized Hb forms on human brain microvascular endothelial cells revealed, that free heme induces cell death whereas oxidized Hb forms in particularly ferrylHb triggers an inflammatory response.

Conclusions: We concluded that RBC lysis, Hb oxidation and heme release play important roles in the inflammatory response following IVH. However, the pathogenic role of covalently crosslinked Hb multimers need to be further investigated.

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Microbial composition and diversity of mice colonized with a carbapenemase-producing *Klebsiella pneumoniae* strain

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Emergence and spread of multidrug-resistant bacteria represent a serious problem worldwide. The extended-spectrum β -lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae (CPE) are in the critical category on the WHO antimicrobial resistance priority list. These bacteria are responsible for a large proportion of hospital-acquired infections. The therapeutic options for CPE are limited, therefore controlling the spread of these bacteria is important. Intestinal colonization plays a crucial role in the spread of CPE.

In this study our goal was to investigate the effect of different per os antibiotic treatments on the microbial composition and diversity of C57BL/6 mice colonized with *K. pneumoniae* (KP5825). This strain expressed a carbapenemase gene, a PMQR gene and an ESBL gene. We also examined the colonization rate and the resistance gene copy number changes in the colonized mice.

All experiments were carried out using 6–8 weeks-old C57BL/6 male mice. For colonization, 5×10^6 CFU of KP5825 was administered orally on the 14th and 15th days of ampicillin pretreatment. After the colonization mice were divided into 6 groups. These groups received the following antibiotic treatments for 15 days: 0.5g/L ampicillin (AMP), 0.1g/L ceftazidime (CAZ), 0.5g/L ceftazidime, 0.1g/L ciprofloxacin (CIP) and 0.5g/L ciprofloxacin was administered in the drinking water (6th group: control). Fresh fecal pellets were collected from the animals every 3 days throughout the 15 days period. From the fecal samples microbial DNA was extracted.

In the second phase of our experiments we used qPCR technique to determine the KP5825 DNA quantities and assess the resistance gene copy numbers. The V3-V4 region of the 16S rRNA gene was amplified and sequenced with the Illumina MiSeq platform to determine the microbiota composition and diversity in the intestines of colonized mice.

Our results show that AMP and CAZ increased the colonization KP5825 and reduced the gut microbiome diversity originally characterized by Bacteroides dominance. In contrast, CIP did not increase the colonization rate. We also observed strong negative correlation between the presence of Lachnospiraceae NK4A136 group 11319, Roseburia, Anaerostipes, Lachnospiraceae 11308, Lachnospiraceae UCG-004, Tyzzerella, Agathobacter, Lachnospiraceae NK3A20 group 11318 genera and the colonization rate with KP5825.

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