CD163 in Neurons: Utilizing a Novel Drug Delivery System to Assess Therapeutic Potential

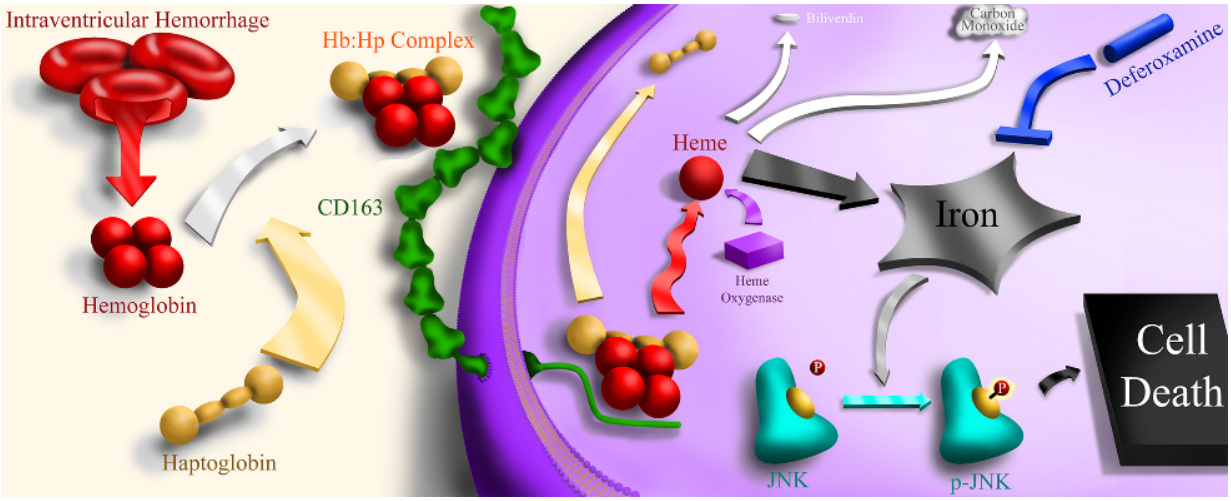

Figure 1: The author's depiction of the Hemoglobin-CD163 scavenging system as it pertains to the events following IVH. The cascade depicts the release of hemoglobin (red) from erythrocytes following IVH, followed by scavenging of this hemoglobin by haptoglobin dimers (yellow). This complex is then endocytosed by CD163 (green) before being broken down into heme (red). This heme is further degraded by heme oxygenase (purple) into iron (grey), which induces activation of JNK to p-JNK (light blue). This leads to apoptotic cell death.

Intraventricular hemorrhage (IVH) is defined as the presence of bleeding into the ventricular system of the brain, most frequently observed in preterm neonates. It is associated with high mortality and morbidity, with almost all infants that survive IVH developing significant cognitive deficits. For over four years, since the summer between my Junior and Senior years of high school, I have investigated potential therapeutic targets for attenuating IVH-induced "secondary injury," or the injury that arises not from the initial hemorrhagic ictus but rather from prolonged biomolecular and physiological events. Some of the prominent agents of this secondary injury are specific blood components that get released into the ventricular system, such as erythrocytes and their contents, coagulation components, leukocytes, and more.[1,2] Of primary interest to me are the events following the hemolysis that occurs during IVH. This erythrocyte lysis releases large quantities of hemoglobin (Hb) into the ventricular system. Using a neonatal rat model of IVH, we demonstrated that this free Hb within the ventricular system significantly damages the nearby hippocampal region, as well as causes significant induction of hydrocephalus.[3] It does so via the ferrous (Fe^{2+}) cations it contains within its heme moieties. These cations have the ability to engage in Fenton reactions as shown below:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^*$$

This reaction produces a hydroxyl radical (OH*), which can subsequently react with a variety of other endogenous substrates to produce widespread oxidative damage. This damage is most potent when iron is allowed to accumulate within cells, and I am therefore interested in identifying pathways by which this hemoglobin-bound iron gains access to cells of interest (namely neurons). Toward this end, I led a study that specifically investigated the mechanism by which iron was inducing cell death in hippocampal pyramidal neurons following IVH.[4] Using immunohistochemical (IHC) and SDS-PAGE techniques, I identified the c-Jun Kinase (JNK) apoptotic cascade as being a significant mediator of the iron-induced cell death. The JNK cascade, however, is intracellularly based, so I needed to identify the mechanism by which the hemoglobin-contained iron was crossing the cell membrane. Using double-labeling immunofluorescence, I identified a hemoglobin-receptor CD163 as being colocalized to JNK activation, indicating it as a prominent pathway for iron infiltration. However, I noticed that this colocalization appeared to occur on cells that were morphologically similar to neurons, a conjecture that was quickly corroborated by subsequent CD163-NSE colocalization (NSE = Neuron-Specific Enolase, a marker for neurons) and RT-PCR in primary neuronal cell cultures incubated in excess

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hemoglobin. The intriguing element was that CD163 has long thought to be expressed solely in monocytes and macrophages, to the extent that it is viewed as a marker for that cell lineage. It is beneficial in those cell lines, as any hemoglobin scavenged by those cells can be properly degraded and the iron sequestered safely. Neurons, however, lack the adequate iron homeostatic systems (namely sufficient Ferritin expression) that monocytes and macrophages contain (Figure 1). My discovery of neuronal CD163 expression was therefore novel and implicated CD163 as a potential therapeutic target. Recently, my findings were independently verified,[5] and our own lab has performed a more comprehensive study demonstrating CD163's expression in cortical and pyramidal neurons (which will be submitted in the winter of 2016-2017).

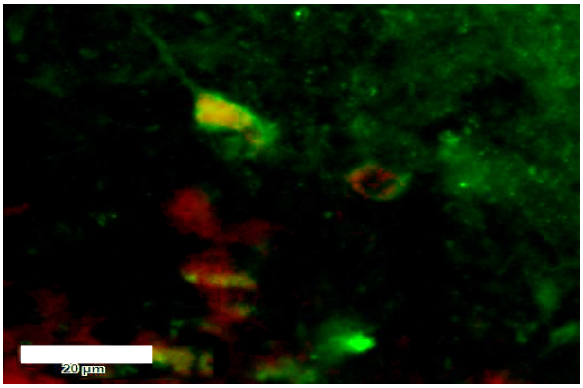

Figure 2: Double-labeling of CD163 (in red) and NSE (in green) showed co-localization of CD163 and NSE in neurons after IVH, indicating neuronal expression of CD163 (40x magnification; scale bars = 20 µm).

Looking forward, I am attempting to identify a method by which we can target and suppress neuronal CD163 following hemorrhage in order to disrupt iron's access to vulnerable neurons. The technique would have to be very specific to neurons, given the receptor's beneficial qualities in non-neuronal cells, and would have to suppress CD163 expression in the brain, which is separated from the rest of circulation by the formidable blood-brain barrier. I have drawn inspiration from the recent innovations in cancer therapy research dealing with targeted nanoparticles.[6] My current plan is to synthesize a peptide-based nanoparticle carrier for CD163 siRNA (a 21-nucleotide RNA sequence complementary to the mRNA that codes for the protein). This nanoparticle would necessarily consist of both a targeting peptide (something that would specifically bind to and be endocytosed by neurons), and an intracellular trafficking peptide (to allow the siRNA maximally efficient release from the endosome following targeted binding) as well as having an increased ability to permeate the blood-brain barrier. I have chosen a peptide component consisting of the targeting glycoprotein from the rabies virus (a virus with excellent ability to bypass the barrier and specifically bind to neuronally-expressed acetylcholine receptors) conjugated at the N-terminal with the intracellular trafficking peptide transportan. By incorporating the 5-FAM (5-Carboxyfluorescein) fluorescent label into this peptide, it is possible to identify its expression using flow cytometry. This protein component would allow distal administration (intravenous instead of direct intracerebroventricular injection), and direct targeting of neuronal AChR. Following this, the nanoparticle would undergo endocytosis and release the siRNA for CD163, thus silencing future expression of the harmful protein in neurons selectively (Figure 3). A similar method has been described in a hypothetical simulation of traumatic brain injury, where the apoptotic agent caspase-3 was silenced using such a nanoparticle system.[7]

I am currently performing *in vitro* experiments using PC-12 neuroblastoma cells (which can differentiate into neuron-like cells following neuronal growth factor NGF administration) and assessing the presence of CD163 via RT-PCR and electrophoresis. The second phase of the project will be administering CD163 siRNA using the standard transfecting agent Lipofectamine 2000 (without nanoparticle assistance) in order to assess the siRNA's efficacy and effect duration. Subsequently, I will synthesize the siRNA-containing nanoparticle, combine it with the siRNA, and test uptake and efficacy *in vitro* using this new drug delivery system.

Once firmly established as viable *in vitro*, a neonatal rat model will be created that will simulate IVH using intraventricular injection of hemoglobin. As in my previous studies, T2-weighted MRI will be used to assess any confounding variables such as post-hemorrhagic hydrocephalus following the administration of the nanoparticles *in vivo*. Neurobehavioral examinations of the rats will be performed to assess any long-term impacts of the treatment. This study will be a multi-year project, and as such

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I am prepared to pursue it into graduate education if necessary. I am planning on developing an R21 grant with Dr. Jennifer Strahle at Washington University in St. Louis in order to fund further exploration into this nanoparticle delivery system and other CD163-related therapy-centered studies. In addition to its potential to investigate a novel hemorrhagic therapy delivery system, this research has the potential to solidify the recent novel finding of neuronal CD163 and illustrate the negative impact this has on neuronal recovery from IVH. It additionally could identify a therapeutic pathway to diminish the dramatic brain injury caused following IVH and thereby improve the prognosis and diminish the mortality rates for the preterm infants that suffer from this condition.

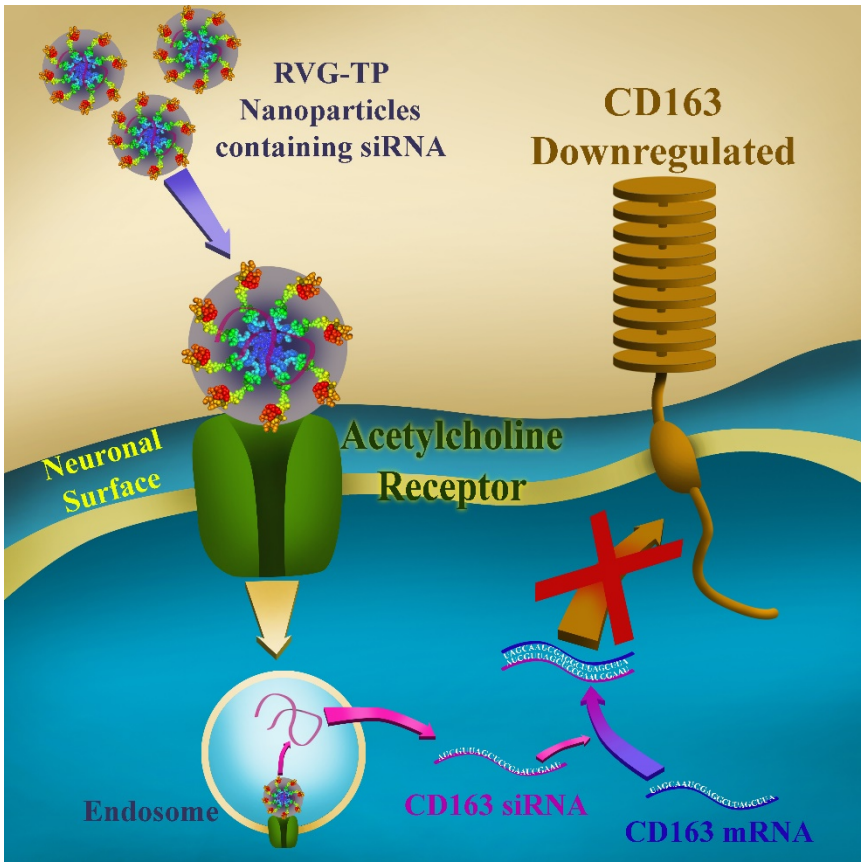

Figure 3: The author's depiction of the nanoparticle-siRNA drug delivery system and its intended effect on CD163 expression. The Rabies Virus Glycoprotein (RVG)-Transportan (TP) nanoparticles containing the CD163 siRNA would bind to AChR on neuronal surfaces. Following endocytosis, the siRNA would be released with the aid of TP (not shown). The siRNA would subsequently bind CD163 mRNA, downregulating synthesis of CD163 on neurons immediately following hemorrhage.

References

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