Title: Effect of the HDAC inhibitor - sodium butyrate on complement system activity in rat model of neonatal hypoxia-ischemia.

Authors: Karolina Ziabska, Halina Zajac, Teresa Zalewska, Malgorzata Ziemka-Nałecz

NeuroRepair Department, Mossakowski Medical Research Institute, Warsaw, Poland

Abstract:

Neonatal hypoxia-ischemia (HI) increases the risk of mortality and life-long neurologic sequelae. HI triggers a series of cellular and biochemical pathways that lead to neuronal injury. It is generally accepted that one of the crucial pathogenic factor in the perinatal brain injury is inflammation. One of the inflammatory component which activates after HI is complement system. Complement activation products consisting of opsonins, anaphylatoxins, and a terminal cytolytic complex mediate its effector functions such as development of inflammatory reaction, clearance of dead and dying cells and post-ischemic remodeling of the synaptic network. The complement system can be a potential therapeutic target for the reduction of brain damage and improvement of outcome after HI. Our recent studies in an animal model of HI revealed the neuroprotective, anti-inflammatory properties of sodium butyrate (SB), a histone deacetylase inhibitor (HDACi). The present investigation was designed to assess whether SB effects on complement activation and synapse elimination after experimental HI.

Seven-day-old rat pups were subjected to unilateral carotid artery ligation followed by 60 minutes of hypoxia (7.6% O_2). SB (300mg/kg) was administered in a 5-day regime with the first injection given immediately after hypoxic exposure. Double immunohistochemical stainings: C3/PSD95; C3/synaptophisin and C5/PSD95 were used to determine the colocalization of complement and synaptic proteins. The expression of complement proteins (Cq1a, C3, C5, C9) was determined by qPCR . The level of the synaptic proteins (synaptophisin, PSD 95) was assessed by Western blot.

The obtained results showed that complement proteins colocalized with the synaptic proteins, however C3 and C5 were present in almost whole investigated brain tissue of control and hypoxic-ischemic animals. The qPCR investigations showed that HI increased the expression of complement proteins in rat brains mostly at 24h time point. The application of SB significantly inhibited the complement system activation. We also observed the decreased level of PSD95 at 3^{th} and 5^{th} day after HI. The treatment of hypoxic-ischemic animals with SB restored the expression of PSD95 to the levels characteristic for control animals.

Obtained results suggest that SB inhibits the complement system activation after HI. This inhibition is correlated with the restoration of the synapses degraded after HI. These findings provide the basis for clinical approaches targeted at protecting the newborn brain damage.